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Enhancing pond productivity: Evaluating performance of Boirali (*Barilius barila*) in polyculture system in farmers' pond of northern Bangladesh

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Abstract

An experiment was conducted in farmer's ponds located in the northern region of Bangladesh from April to September 2022 to observe the production performance of Boirali, *Barilius barila* under polyculture system in different stocking densities. A mixed pattern trial of Boirali, Indian major carp, Catla, Silver carp, Silver barb and GIFT was conducted with three treatments each with three replications in seasonal ponds at six Upazilas under Rangpur division. Three different stocking densities of Boirali *viz.,* 500, 600 and 700 per decimal with 12 Catla, 6 Silver carp, 10 Silver barb and 8 GIFT in each treatment maintained in T_1, T_2 and T_3 respectively. The average final weight of Boirali after 150 days of culture were 13.7±1.20, 16.4±1.4 and 14.8±1.10 g in T_1, T_2 and T_3 respectively where initial average weight was 1.25±0.3g. The highest length and weight of T_1, T_2 and T_3 were found 11.9±0.2, 13.8±0.34,12.7±0.11cm and 14.9±0.3, 17.8±0.1, 15.9±0.2g respectively. Specific growth rate (1.71±0.21) and feed conversion ratio (2.54 ± 0.01) were found significantly higher in (T_2) compared to other treatments. Among the three treatment, T_2 shown a considerably ($p<0.05$) greater benefit-cost ratio (1.57±0.04), total fish production (5627±220.3 kg ha^{-1}) and production of boirali (2321±77.5 kg ha^{-1}) after 150 days of culture. The stocking densities of 600 Boirali per decimal (T_2) with other fishes is the most suitable to get highest production under polyculture system.
Key words: *Barilius barila*, Borali, Polyculture

Introduction

Aquaculture is the most promising sector for protein intake for the people of Bangladesh. It is widely believed that polyculture increases the productivity of the aquaculture system by efficient utilization of ecological resources within the environment (Wahab *et al.* 2011). Stocking two or more complimentary fish species can increase the maximum standing crop of a pond by utilizing a wide range of available food items and the pond volume (Wahab *et al.* 2019). *Barilius barila* is a freshwater indigenous fish species commonly occurring in hilly and river streams of Rangpur,

Dinajpur, Sylhet, Mymensingh, Chandpur, and Chattogram (Rahman 2005). Preliminary survey indicates that the abundance of these species has been gradually decreasing in nature in the last decades. This candidate species contains high nutrient profile and high market demand also (Bogard 2015). There was no available information about Boirali (*B. barila*) on reproductive biology and also on induced spawning, nursing, rearing and intensive culture of these fishes. Recently, Bangladesh Fisheries Research Institute, Freshwater Sub-Station, Saidpur has developed induced breeding and nursery rearing techniques in captive condition of this candidate species. This candidate fish is enlisted as an endangered category by IUCN (2015). But till now, it is not practiced of streamed fish's polyculture or monoculture in farmer's fields and not much research work has been done on it. In the northern districts of Bangladesh about 55% of ponds are seasonal of which 60% retain water for 4-6 months while 40% retained for 6 to 9 months in a year and even more in some areas (Hussein 2019). These small water bodies are being used mainly for household activities but some are still abandoned due to their derelict and marshy nature (Nur and Haque 2021). To ensure proper utilization of seasonal ponds, the culture of others carps those growth rates is fast should be introduced in the semi-arid zone to enhance fish production (Shah 2008 and Hasan *et al.* 2023). From an aquaculture perspective, many water bodies have great potential for fish culture. So Boirali under a polyculture system with other carps those have faster growth and require low inputs are used for this experiment. These species include Catla (*Catla catla*), Silver carp (*Hypophthalmichthys molitrix*), Silvere barb (Sharpunti, *Barbodes gonionotus*), Genetically Improved Farmed Tilapia (GIFT, *Orechromis niloticus*). The demands for those fishes are owing to their taste and medical values. Unfortunately, the culture technologies of Boirali with other carp's species have yet not been optimized and evaluated especially in the northern part of Bangladesh. Hence, the present study has been designed and proposed to demonstrate these research results in the farmer's field as well as to validate the technologies.

Materials and Methods

Experimental design and selection of pond
For this study, a total of nine seasonal ponds have been selected in different Upazilas of Rangpur region. The nine ponds have been divided into three groups. Each group treated as one treatment e.g. treatment-I (T_1), treatment-II (T_2), treatment-III (T_3) and each treatment has three replications (Table 1). The areas of ponds ranged between 12 and 15 decimal and depths between 1.0 and 1.5 m. The culture tenure was 150 days. Water quality parameters and fish sampling data were collected fortnightly.

Table 1. Polyculture pattern of Boirali, *Barilius barila* with other short cycle species

Treatments	Species combination	Stock. Dens. (indi. dec.$^{-1}$)	Fingerlings
T_1	Boirali+Catla+Silver carp+Sarpunti+GIFT	500+12+6+10+8	
T_2	Boirali+Catla+Silver carp+Sarpunti+GIFT	600+12+6+10+8	Over wintered
T_3	Boirali+Catla+Silver carp+Sarpunti+GIFT	700+12+6+10+8	

Pond preparation and stocking of fingerling

Before beginning the experiment, the native and unwanted fishes were removed from the pond by repetitive netting then ponds were completely dried to eradicate unwanted crustaceans/fishes/eggs that remained in cysts. Pond embankments were prepared. Three layers of netting were done *viz.* slope netting, side netting and surface netting for complete restriction of unwanted predators like animals/birds. Pond bottoms were treated with lime at the rate of 1 kg/decimal and left for 3 days. Then the ponds were filled with groundwater and fertilized with Urea and TSP 100g and 75g per decimal respectively. An average of 2-3 cm of Boirali fingerlings and 5-10 cm of other fish species were stocked according to the stocking densities (Table 1) and the Initial length and weight of fingerlings were measured before releasing into the pond.

Feeding and Sampling

Commercial fish feed was fed containing 30-35% protein at the @ 10-5% of body weight twice daily. Length-weight data were collected fortnightly from 8.00 am to 9.00 am to determine the growth rate as well as food adjustment. Fish length was measured using a measuring scale (cm) and weight was taken by digital weight balance (Measuring range 1g to 1000g). Final harvest was done after completion of 5 months of culture.

Analysis of water quality parameters

Water quality parameters such as water temperature (°C), water pH, and Dissolved Oxygen (DO) (mg/L) and Total Dissolved Solid (TDS) (mgL^{-1}) were measured by HANNA (HI98494 pH/EC/DO) multi-meter, and NH_3, Hardness, Alkalinity (mg/L) were measured by HACH kit (Hanna Co. Japan) of the experimental ponds.

Growth parameters formula

Weight gain was calculated as follows:

Weight gain (g) = Final weight (g) - Initial weight (g)

a) Percent weight gain (%) was calculated as follows:

$$\text{Percent weight gain} = \frac{\text{Final weight (g) - Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

b) Average daily gain (ADG % day^{-1}) was calculated as follows:
Average daily gain (ADG % day^{-1}) =

c) Specific growth rate (SGR % per day) was calculated as follows:

$$SGR \% \, day^{-1} = \frac{InW2 - InW1}{T2 - T1} \times 100$$

Where, W_1 is the initial live body weight (g) at time T_1 and W_2 is the final live body weight (g) at time T_2 (day) (Brown 1957).

Feed utilization

a) Feed conversion ratio was calculated as follows:

$$FCR = \frac{Total\,dry\,feed\,intake(g)}{Total\,wet\,weight\,gain(g)} \quad \text{(Castell and Tiews 1980).}$$

Survival rate and production

a) Survival rate (%) was calculated as follows:

$$Survival\,rate\,(\%) = \frac{Final\,number\,of\,fish}{Initial\,number\,of\,fish} \times 100$$

b) Production (kg decimal^{-1}) = $\dfrac{Final\,number\,of\,fish\,harvested \times individual\,weight\,of\,fish}{1000}$

Production (kg ha^{-1}) = $\dfrac{Final\,number\,of\,fish\,harvested \times individual\,weight\,of\,fish}{1000} \times 247.1$

Statistical Analysis

Data were analyzed using MS Excel and one-way analysis of variance (ANOVA) (Duncan 1993) and SPSS 20 (Chicago, USA) to detect significant differences among the treatments at a 5% level. The values were given with means ±SD, and differences were considered significant at the subset for alpha = 0.05 ($p \leq 0.05$).

Results

Water quality parameters of experimental pond

Water quality parameters such as Water temperature(°C), pH, Dissolved oxygen (mgL^{-1}), Ammonia (mgL^{-1}), Total Dissolved Solids (mgL^{-1}), Hardness (mgL^{-1}) and Alkalinity (mgL^{-1}) was measured during culture period of selected farmer's ponds are presented in Table 2, where water temperature was ranged between 26-31.2°C, pH was 7.2-7.8 , DO (mgL^{-1}) was 5.3-6.05, Ammonia was 0.07-0.14, TDS (mgL^{-1}) was 65.9-95.6, Hardness was 98.5-116 and Alkalinity was 96.2-12-.5   of experimental pond during 150 days of culture period.

Table 2. Water quality parameters of the experimental ponds

Parameters	T_1	T_2	T_3
Water Temp. (˚C)	29.0±2.2	28.8±2.0	28.3±2.3
Water pH	7.4±0.20	7.5±0.24	7.7±0.10
DO (mgL^{-1})	6.0±0.5	5.5±0.2	5.4±0.15
Ammonia (mgL^{-1})	0.11±0.03	0.08±0.01	0.10±0.02
Total Dissolved Solids (mgL^{-1})	70.4±4.5	88.3±7.3	76.6±6.4
Hardness (mgL^{-1})	111.4±4.4	112.22±3.8	103.2±4.7
Alkalinity (mgL^{-1})	112.4±7.65	102.5±6.3	108.4±7.2

After 150 days of culture, the growth performances of *Barilius barila* under poly-culture in farmer's ponds are shown in Table 3.

Table 3. Growth and Production of *Barilius barila* under polyculture

Parameters	Treatments		
	T_1	T_2	T_3
Stock. Dens. (indi.dec^{-1})	500	600	700
Initial Length (cm)	2.5±0.4	2.5±0.4	2.5±0.4
Initial weight (g)	1.25±0.2	1.25±0.2	1.25±0.2
Final Length (cm)	11.7±0.20[c]	13.6±0.4[a]	12.8±0.12[b]
Final weight (g)	13.7±1.20[c]	16.4±1.4[a]	14.8±1.10[b]
Weight gain (g)	12.45±1.30[c]	15.15±1.28[a]	13.55±1.20[b]
SGR bw (% day^{-1})	1.59±0.23[c]	1.71±0.21[a]	1.64±0.20[b]
ADG bw (g day^{-1})	0.13±0.01	0.17±0.02	0.15±0.01
Production of Boirali (Kg/ha) after 150 days of culture	2022±47.2[b]	2321±77.5[a]	2198±54.4[c]
Production of fishes (Kg/ha)	4689±161.2[c]	5627±220.3[a]	5388±164.5[b]
FCR	2.66 ± 0.01[b]	2.54 ± 0.01[a]	2.78 ± 0.02[c]
Survival rate (%)	89.1 ± 3.1[a]	84.04 ± 3.7[b]	87.1 ±2.6[c]

Values with different letters within each row represents significant ($p < 0.05$) difference

Growth and yield performance of Barilius barila in different treatments under polyculture system

The growth performance of *Barilius barila* was measured by after analysis of some growth parameters such as Specific growth rate (% day^{-1}), Average daily growth (% day^{-1}) on body weight basis, total production of Boirali (Kg/ha), total production of fishes

(Kg/ha) after 150 days of culture was measured. Feed conversion ratio and survival rate was also measured for economic viability and cost benefit analysis. The following Fig. 1 and Fig. 2 showed the increase of growth rate of *Barilius barila* in farmer's pond during 150 days of culture.

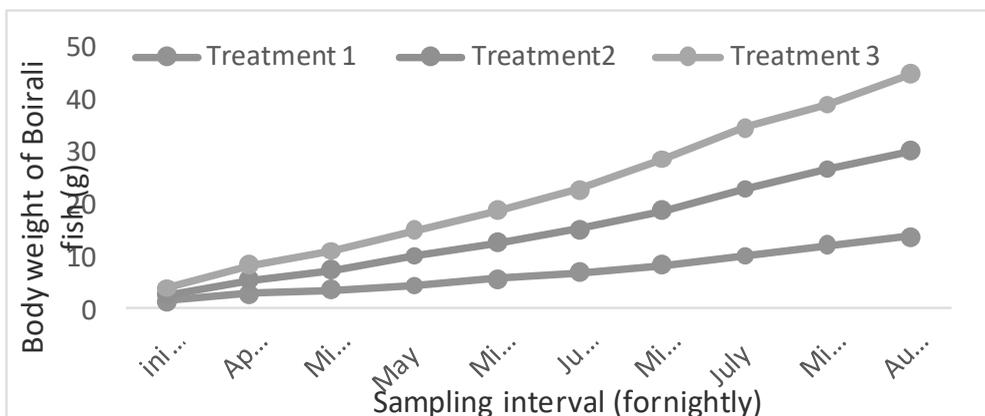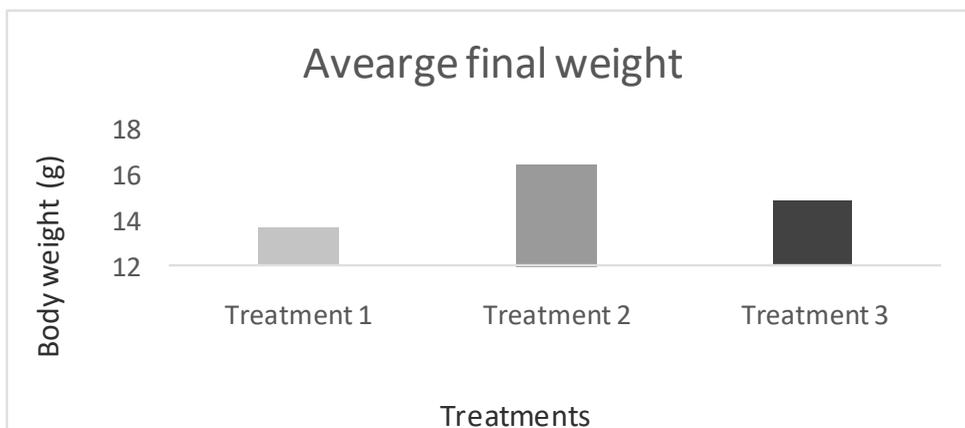**Fig. 1.** Increasing growth pattern of *Barilius barila* at 15 days interval.

Fig. 2. Average final body weight of *Barilius barila* fish in different treatment.

Growth and yield performance of other carp's fishes in polyculture

The initial and final body weight of other associated fish species which cultured with Boirali (*Barilius barila*) under polyculture system are presented in Table 4.

Table 4. Body weight of other fish species under polyculture

Species	Initial weight(g)	Final body weight(g)		
		T_1	T_2	T_3
Catla	15.8±2.86	331.33±18.69	374.5±25.58	343.5±23.4
Silver carp	15.6±2.54	357±21.31	393.11±27.31	362.1±22.8
Sarpunti	14.4±1.14	183.13±16.5	245.48±17.24	189.13±17.5
GIFT	13.13±0.92	307.23±19.2	365.33±22.11	312.3±18.8
Survival rate (%)		96.02 ± 1.7[a]	94.06 ± 2.2[b]	90.04 ± 1.3[c]

Economic viability of Barilius barila in polyculture

Economic viability of *Barilius barila* culture under different treatments with other fish species in farmer's pond is presented in Table 5.

Table 5. Benefit and cost analysis of *Barilius barila* under polyculture in different treatments

Item wise expenditure	T1	T2	T3
Pond preparation (Tk ha^{-1})	30,000	30,000	30,000
Fingerling cost (Tk ha^{-1})	2,30,000	2,65,000	2,90,000
Lime and fertilizer (Tk ha^{-1})	12,500	12,500	12,500
Feed cost (Tk ha^{-1})	7,05,375	7,75,830	8,45,400
Transport, labor etc. (Tk ha^{-1})	50,000	50,000	50,000
Total production costs (Tk ha^{-1}) after 150 days of culture	10,27,420 ± 1488[c]	11,33,330 ± 1624[b]	12,27,900 ± 1860[a]
Income and output	T1	T2	T3
Total production (Tk ha^{-1})	4689.6±18.03	5627.5±25.04	5388.8±21.40
Gross return (Tk ha^{-1})	14,94,700 ± 104[c]	17,84,500 ± 203[a]	16,84,300 ± 226[b]
Gross margin (Tk ha^{-1})	4,67,280± 3195[b]	6,51,170± 4690[a]	4,56,400± 3508[b]
Benefit cost ratio	1.45±0.03[b]	1.57±0.04[a]	1.37±0.02[c]

Within rows values with different superscripts are significantly different (p< 0.05)

Discussion

Water quality parameters of the experimental ponds
The water temperature varied between 26-31.2°C during the experimental period of 150 days. There was no significant ($p<0.05$) difference among the treatments in case water temperature. The values of water temperature are similar those reported by (Kohinoor *et al.* 2004 and Hasan *et al.* 2023), where they found 26-30°C suitable temperature for congenial aquaculture. The pH was 7.4, 7.5 and 7.7 in T_1, T_2 and T_3, respectively. The pH

range was found to be suitable in the present study which agrees well with the findings of (Swingle 1995, Mou *et al*. 2018), where they found pH 7.5 to 8.2 in the culture waterbodies. The DO concentration ranged from 5.3-6.05 mgL^{-1} during the experiment and no significant difference was observed among the treatments. Analytical results showed the pH and DO values were inversely related to the stocking density (Chakraborty *et al.* 2005). The ammonia varied from 0.07-0.14 mgL^{-1} among the treatments. Ammonia was slightly higher in (T_3) due to higher stocking densities of fishes, which also proved the statement said by (Hasan *et al.* 2023), where they found 0.13 mgL^{-1} in case of Tengra 700 ind./decimal. Alkalinity was 112, 102 and 108 mgL^{-1} in T_1, T_2 and T_3, respectively in this experiment. Alkalinity was also in suitable range of 105-118 (mgL^{-1}) in the experimental pond. Total alkalinity of more than 100 mgL^{-1} should be present in highly productive water bodies (Alikunhi 1957) which was similar to this finding from the above discussion it can be said that water quality parameters were observed in congenial for fish culture.

Polyculture of Boirali in farmer's pond in different treatments

In this experiment, the average body weight of Boirali was 13.7, 16.4 and 14.8 g in T_1, T_2 and T_3 respectively. The highest weight gain (15.15g) was found in T_2 and the lowest (12.45 g) was found in T_1. The weight gain of Boirali was found significantly ($p< 0.05$) higher in T_2 followed by T_1 and T_3. The results showed that higher growth rate was observed at medium stocking densities. The SGR of Boirali on the basis of body weight were 1.59 ± 0.23^c, 1.71 ± 0.21^a and 1.64 ± 0.20^b % day^{-1} in T_1, T_2 and T_3, respectively and significantly ($p<0.05$) different from each other.

The FCR values were higher in T_3 (2.78 ± 0.02^c) and lower in T_1 (2.54 ± 0.01^a). FCR value directly related to stocking densities showed by analytical values. Hasan *et al.* (2023) also found that small indigenous species have similar FCR value. They found higher FCR value in T_3 (2.70) which contain 700 ind./dec. and lower in T_1 (2.15) which contain 500 ind./dec. in case of tengra (*Mystus vittatus*) under polyculture system. The survival rate was estimated after harvest of fish at the end of the five-month of culture period. Survival rate of Boirali were 89.1 ± 3.1^a, 84.04 ± 3.7^b and 87.1 ± 2.6^c % in T_1, T_2 and T_3 respectively which were significantly ($p<0.05$) different from each other. The results are more or less similar to Kohinoor *et al.* (2004) who found in case of Gulsha (*Mystus cavasius*) fish where the survival rate was 89,85 and 79% in case of T_1, T_2 and T_3 respectively. Stocking density is known to be one of the important parameters in the fish culture which directly affects growth and survival rate of fish (Ahamed *et al*. 2017). Based on analytical values the production of Boirali and total production of all fishes in T_2 was found to be significantly ($p<0.05$) higher followed by T_1 and T_3. In another experiment, Kohinoor *et al.* 2005 said that the highest production was obtained where

the stocking density was the minimum and vice versa in the case of bata (*Labeo bata*) fish polyculture. Where the bata fish production was 2,466±78, 2,395±88 and 2,074±71 kg/ha was obtained from T_1 (5000 ind/ha), T_2(7500 ind/ha) and T_3 (10000 ind./ha) respectively after six months. In this experiment, production of Boirali fish and other fishes' production was significantly influenced by different stocking densities. The analytical results consistently demonstrated that within the treatments, 600 Boirali per decimal (T_2) with other fishes exhibit higher production level for Boirali, total fish yield, and Benefit-Cost Ratio (BCR).

Economic analysis of polyculture of Barilius barila

Fingerlings and feed were the main investment and the profit was calculated by subtracting the expenditures on pond management from the value of harvested fish (Kunda *et al.* 2014). The gross return value (Tk./ha/yr) was 14,94,700 ± 104[c], 17,84,500 ± 203[a] and 16,84,300 ± 226[b] in T_1, T_2 and T_3, respectively with significant difference ($p<0.05$) among the treatments. Furthermore, the gross margin (Tk. ha^{-1}) was 4,67,280± 3195[b], 6,51,170± 4690[a] and 4,56,400± 3508[b] in T_1, T_2 and T_3, respectively which were significantly ($p<0.05$) different. The highest BCR was achieved in T_2 (1.57±0.04[a]) followed by T_1 (1.45±0.03[b]) and T_3 (1.37±0.02[c]) which were significantly ($p<0.05$) different. The production in the present study were more or less similar with the findings of Kohinoor *et al.* (2004) who stated that the production of gulsha, *Mystus cavasius* in polyculture varied between 4050 and 4650 kg ha^{-1} in 06 months' culture period. Hasan *et al.* (2023) studied on tengra, *Mystus vittatus* and found highest BCR was (1.60) in T_1 followed by T_2 (1.31) and T_3 (1.20) which matches our present study findings. Based on total production and BCR it can be concluded that the stocking densities of 600 Boirali per decimal (T_2) with other fishes was the best combination for Boirali polyculture in seasonal ponds at the semi-arid zone of Bangladesh.

Polyculture of Boirali (*Barilius barila*) in seasonal waters within the semi-arid zone has proven to be economically viable. After considering growth and survival rate, a stocking density of 600 individuals per decimal of Boirali was identified as optimal for the polyculture system. The recommended combination for successful Boirali polyculture includes 600 Boirali, along with 12 Catla, 6 Silver carp, 10 Sarpunti, and 8 GIFT individuals per decimal. The ideal culture period from April to September, focuses on overwintered fingerlings for successful culture.

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Production performance of freshwaters mud eel *(Monopterus cuchia)* culture using live feed in Chattogram Hilly Districts, Bangladesh

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Abstract

The study evaluated the growth and productivity of freshwater mud eel (*Monopterus cuchia*) in three districts e.g. Rangamati (S_1), Bandarban (S_2) and Khagrachhari (S_3) of Chattogram hilly region of Bangladesh for a culture period of 6-month from March to September 2023. Fingerlings were supplied live feed, such as small indigenous species at 1.5% body weight every three days' interval and live vermi worm at 1.5% body weight every day in all cultured ponds. Juvenile cuchia were stocked at 10 nos/m² in each pond having mean initial body weight of 50-60g and mean initial body length of 25-34 cm where highest weight gains of 126.55±2.8 g was found in S_3 (*p* < 0.05), followed by the S_2 (116.57±1.89 g) and S_1 (109.09±2.37 g). Survival rate (74.7±2.7 %) and specific growth rate (0.70±0.02 % day^{-1}) were also found to be higher in S_3 compared to the S_1 and S_2. Over a 6-month culture period, net production was found 4550.46±83.2 kg in S_3, 3929.39±65.57 kg in S_1 and 4180.42±78.5 kg in S_2. The production performance of Cuchia in hill tract districts was encouraging by using live small indigenous species and vermi worm.
Keywords: *Monopterus cuchia*, Cuchia, Hilly districts, Vermi

Introduction

The freshwater mud eel, *Monopterus cuchia* is a freshwater air-breathing fish commonly found in freshwater and brackishwater with an altitudinal range of 76–1350 m above sea level (Nahid *et al*. 2020). It has historically been used to treat diabetes, hemorrhoids, piles, asthma, anemia, and weakness (Narejo *et al*. 2003a). Due to its nocturnal and carnivorous feeding habits, fish prefer food that comes from animals, such as small fish, amphibians, crustaceans, insect larvae, echinoderms, aquatic invertebrates, earthworms, snails, tubifex, fish fingerlings, insect pupae (Abedin *et al*. 2020, Nahid *et al*. 2020). Furthermore, during daytime they usually hide under mud and stones depicting their adaptability to various environmental conditions.

Bangladesh is home to four different species of eels: *Monopterus cuchia*, *Anguilla bengalensis*, *Pisodonophis boro*, and *Pisodonophis cancrivorus* (Karim 1978, Anon 1980, Chowdhury *et al.* 1980, Raja 1980, Hussain 1982, BOBP 1985). In Bangladesh, the mud eel has emerged as a viable and exportable fish species, with aquaculture and research potential. But the number of this species in nature has recently dropped due to strong fishing pressure, habitat destruction, aquatic pollution, and indiscriminate pesticide usage in agriculture and industrial activities, among other factors (Ponniah 2000). It is now considered a vulnerable fish species in Bangladesh (IUCN Bangladesh 2015).

According to the 2022 census, the tribal population is roughly 60% living in the Chattogram hill tract area (BBS 2023). There are around thirteen tribes in three districts, which are divided into about one hundred other sects. Mud eel is primarily consumed by non-Muslims and ethnic groups in Bangladesh. This sector employs around 8,000 fishermen, traders, transportation operators, and exporters. A few research have examined the function of mud eels' shelters (Narejo *et al.* 2003), the effects of temperature on growth, survival, and production (Rahman *et al.* 2005), co-management (Tara 2010), larval and grow-out techniques (Khanh and Ngan 2010), the effects of various feed types (Narejo *et al.* 2011), the performance of rearing and production using alternative feeding rations (Miah *et al.* 2012, Lima *et al.* 2023), and the growth and economics of culture under different stocking densities (Bashak *et al.* 2021). Most of these studies were conducted using various feeds and feeding ratios. Nonetheless, no suitable studies on the production performance of the mud eel culture technique employing live feed have been conducted for the Chattogram hill tract areas. This study may result in the development of possible mud eel cultivation techniques in the Chattogram hill tract areas, which would help the nation's economy, meet the demand for protein, and stimulate growth in the export industry.

Materials and Methods

Experimental site
The experiment was conducted in three hill districts e.g. Rangamati (S_1), Bandarban (S_2) and Khagrachhari (S_3) of Chattogram division (Fig. 1) during the period of March to September 2023. The experiment was executed with three sites having two replications in each district, with the help of the officials of Department of Fisheries. The following design was followed in each site (Table 1).

Table 1. Design of the experiment

Study area	Feed type	Stocking density
Rangamati (S_1) Bandarban (S_2) Khagrachhari (S_3)	Live SIS (1.5% of BW) at 3 days interval and Vermi (1.5% of BW) Every day	10 nos/m^2

Fig. 1. Experimental sites. S_1. Rangamati, S_2. Bandarban, S_3. Khagrachhari.

Pond preparation: The experiment was conducted in six ponds with a water area was 40m^2 each pond. Ponds were dug with (30×15×3.5) ft^3. The pond bottom was covered by polythene, 0.5 cm mesh size knotless nylon net and triple then fill-up with 08–12-inch clay mud. The ponds were prepared by treating the soil with quick lime at the rate of 2kg per decimal. After 15 days, ponds were filled up with 0.6-0.8m water and then dolomite at the rate of 15 ppm to strengthen the water's buffer capacity. After three days, the pond water was fertilized with Urea, TSP and MoP at the rate of 2.5 ppm, 3.0 ppm and 1.0 ppm respectively to accelerate primary productivity. Water hyacinth and PVC pipe were used as shelter for Cuchia. The ponds were protected by fencing with a 0.2 cm nylon net.

Cuchia fingerlings collection and stocking: The Cuchia fingerlings were collected from the Cuchia collectors of the Netrokona and Mymensingh district with an average weight of 50-60 g. Fingerlings were carefully transported in experimental drums with water. Just before release, fingerlings were dipped in a 5% potassium permanganate solution for a few seconds to ensure removing germs present on the fish surface and stocked at the stocking density of 10 nos/m^2 in each pond.

Feeding of Cuchia: After stocking, Cuchia were provided live feed such as available live small indigenous species at 1.5% of body weight every three days' interval and live vermi worm at 1.5% of body weight every day. As they are nocturnal feeders, feed was given after sunset daily at the same place of the ponds in feeding tray. Feeding was done up to their satiation level.

Water quality parameters monitoring: Water quality parameters such as DO, water temperature, TDS, pH, alkalinity, hardness and ammonia were recorded monthly. In situ, water temperature, TDS, dissolved oxygen (DO) and pH were measured using a HANNA series portable multiparameter (HI 98194). Hardness and alkalinity was determined by titrimetric method. Ammonia was measured by using ammonia kit.

Fish growth monitoring: Fish sampling was carried out by collecting PVC pipe shelters or collected from water hyacinth by using triangular shaped small mesh peeling net. Around 10% of fish in each treatment were sampled at one-month intervals to determine length (measuring scale: ±1.0 mm) and weight (TANITA digital scale, model KD-160, Japan). At the final harvest, ponds were drained out, and fish were weighed and measured to determine growth parameters.

The following formulas were used to assess the experimental fishes' growth response, yi eld, and survival rate (Brown 1957).
   a) Weight gain (WG) = Final fish weight (g) - Initial fish weight (g)
   b) Weight gain (%) = (Final weight - initial weight) × 100/ initial weight
   c) Average daily weight gain (ADWG) = (Final weight - initial weight)/ culture days
   d) Specific growth rate (SGR) = 100 × (ln final weight - ln initial weight)/culture days
   e) Feed conversion ratio (FCR) = Feed applied (dry weight)/ Live weight gain
   f) Survival rate (SR%) = 100 × (number of fish survived/number of fish stocked)

Statistical analysis: The mean values of water quality parameters and growth parameters were subjected to one-way ANOVA (Analysis of variance), and DMRT (Duncan's Multiple Range Test) was done by using the SPSS (Statistical Package for Social Science) version 20 (Duncan 1955).

Results and Discussion

The mean levels of water quality parameters (±SE) of culture pond are summarized in Table 2. Although the physicochemical characteristics of water varied between sites over the culture period and did not change significantly among the sites ($p > 0.05$). Water temperature ranged between 26.75 ^{0}C to 27.84 ^{0}C in all sites during the study period,

which supports by similar result study (Lima *et al*. 2023). According to Boyd (1982), tropical fish culture requires water temperatures ranging from 26.06 to 31.97 ^{0}C. DO and pH concentration, alkalinity, and ammonia were higher in treatment S_2, while lower results were recorded in S_1, but within the acceptable range in all sites. Oxygen is the most important stress factor and should be higher than 5 mg/L for optimal growth of cultured fish species. In addition, the acceptable range for fish culture is normally between pH 6.5-9.0. The results revealed that dissolved oxygen and pH concentrations were 5.6-6.18 mg/L and 7.37-8.1, respectively. Rahman *et al*. (2005), Tara (2010), Begum *et al*. (2018), Chowdhury *et al*. (2019), Das *et al*. (2022) and Lima *et al*. (2023) found more or less similar results in Cuchia culture pond in different areas. According to Boyd (1990), the total alkalinity of productive ponds should be 20 ppm or above, and the higher the total alkalinity, the higher the fish productivity, which supports the present findings.

Table 2. Mean value (± SE) of water quality parameters of different treatments

Water quality parameters	S_1	S_2	S_3
Water temp. (^{o}C)	27.84±1.09 [a]	27.2±1.6 [a]	26.75±1.7 [a]
DO (mg/l)	5.6±0.7 [a]	6.18±0.4 [a]	6.08±0.8 [a]
pH	7.37±0.4 [a]	8.1±0.6 [a]	7.48±0.47 [a]
TDS (mg/l)	39.5±2.5 [a]	36.0±3.2 [a]	33.4±2.3 [a]
Alkalinity (mg/l)	68.1±1.7 [a]	73±2.6 [a]	59.2±1.5 [a]
Ammonia (mg/l)	0.1±0.01 [a]	0.2±0.01 [a]	0.1±0.01 [a]
Hardness (mg/l)	53±1.01 [a]	55±2.05 [a]	65.7±2.45 [a]

Mean values with same superscripts in the same row do not differ significantly ($p > 0.05$).

The growth performance of *Monopterus cuchia* of six-month cultured period is summarized in Table 3. The survival rate of Cuchia in the present experiment was found to be 70.2±3.11%, 70.85±0.49%, and 74.7±2.7% in S_1, S_2, and S_3 respectively, with no significant differences among the treatments ($p > 0.05$). In this experiment, the Cuchia survival rate was very similar to that of Khan *et al.* (2021) study for the same species raised in an earthen pond at 400 fry/decimal stocking densities utilizing various diet types. Narjeo *et al.* (2003) found a 70% survival rate in PVC pipe, whereas Khanh and Ngan (2010) found a 73.1-73.5% survival rate in high stocking densities, which was more similar to the present findings. In contrast to the current data, Miah *et al.* (2015) found that the survival rate of this species was 92.5% when raised in clay ditches with live fish. The lower survival rate of present study could be due to the escape of mud eel from the culture ponds.

Table 3. Production performance of *Monopterus cuchia* in different treatments

Growth Parameters	S_1	S_2	S_3
Initial length (cm)	33.9±4.05 [a]	34.49±2.71[a]	33.46±3.67 [a]
Final length (cm)	52.2±5.7 [c]	55.45±6.6 [b]	58.7±6.2 [a]
Initial weight (g)	50.66±5.38 [a]	52.08±5.05 [a]	50.0±4.99 [a]
Final weight (g)	159.75±7.05 [c]	168.65±8.5 [b]	176.5±7.6 [a]
Weight gain (g)	109.09±2.37 [c]	116.57±1.89 [b]	126.55±2.8 [a]
Weight gain (%)	215.34±2.3 [c]	223.83±1.64 [b]	253.0±2.5 [a]
Average daily weight gain (g)	0.61±0.02 [c]	0.65±0.02 [b]	0.70±0.01[a]
Specific growth rate (SGR)	0.63±0.3 [c]	0.65±0.01[b]	0.70±0.02 [a]
Feed conversion ratio (FCR)	3.01±0.1 [a]	2.72±0.22 [a]	2.35±0.5 [b]
Survival rate (%)	70.2±3.11 [a]	70.85±0.49 [a]	74.7±2.7 [a]
Net Production (kg/acre/6 months)	3929.39±65.57 [c]	4180.42±78.5 [b]	4550.46±83.2 [a]

Mean values with different superscripts in the same row differ significantly ($p < 0.05$).

In the current study, the mud eel's growth was higher in S_3 and lower in S_1 in terms of final length, final weight, weight gain (g), and specific growth rate (Table 3). Additionally, during the culture period, S3 showed a higher increasing trend of weight gain than S1 and S2 (Fig. 2). A significant difference ($p<0.05$) was observed between the average weight gain of 109.09 g achieved in the S_1 treatment, which grew steadily at a rate of approximately 0.61 g day^{-1} throughout the culture period. The average weight gains in the S_2 and S_3 treatments was found to be 116.57±1.89 g and 126.55±2.8 g, respectively (Table 3). According to Khan *et al.* (2021) the Cuchia's average daily weight gain fell between 1.14 to 1.29 compared to the same species raised in clay ponds with varying diet kinds, which was comparatively higher than the present study.
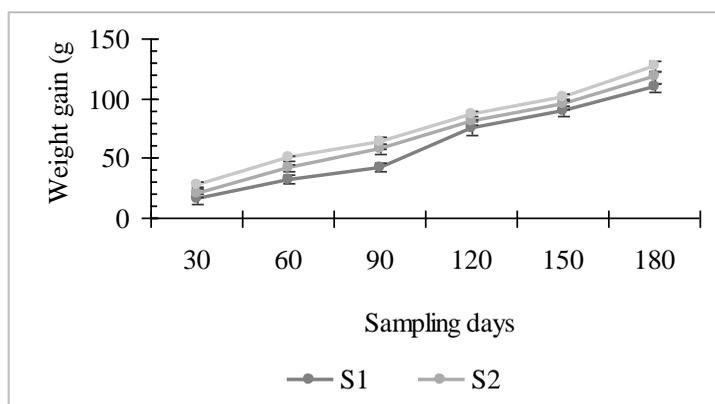

Fig. 2. Growth of Cuchia in different sites during the experimental period.

Specific growth rate (SGR) of Cuchia ranged from 0.63 to 0.70 and was substantially greater in S_3 ($p<0.05$) than S_2 and S_1. The SGR of Cuchia in the current study was similar than that reported (0.64 to 0.79% day^{-1}) in different diches conditions and utilizing different types of food in an earthen pond (Hosen *et al*. 2019, Khan *et al*. 2021, Lima *et al*. 2023). The FCR value in the present experiment was found to be 3.01±0.1, 2.72±0.22 and 2.55±0.5 in S_1, S_2, and S_3 respectively, with significant differences in site S_3 ($p< 0.05$). The FCR value in this study was more similar (2.27 to 2.61) to that reported by Jahan *et al*. (2020) for domesticated species in clay ponds and Lima *et al*. (2023) in Rangamati and Khagrachhari district by using various feed ingredients (2.45 to 3.09). The production performance in the present study was entirely satisfactory after six months of observation. Over a 6-month culture period, the net production of Cuchia in ponds from S_1, S_2, and S_3 were 3929.39±65.57 kg; 4180.42±78.5 kg and 4550.46±83.2 kg respectively. Lima *et al*. (2018) and Das *et al*. (2022) observed more or less similar results by using different types of feed. Results from the present study indicate that live fish and vermi worm was suitable for the growth performance of *M. cuchia* in hill tract districts for its better growth, survival rate and production.

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Production performance of *Monopterus Cuchia* culture in Chattogram Hilly Districts, Bangladesh

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Evaluation of growth performances and economic viability of Shing (*Heteropneustes fossilis*) in biofloc aquaculture system

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Abstract

Biofloc technology (BFT) is an eco-friendly aquaculture technique based on in situ microorganism production. This study was carried out during April to September 2020 to find the feasibility assessment of *Heteropneustes fossilis* culture in biofloc system in Northern region of Bangladesh. Three different stocking densities of *H. fossilis* were introduced in three tanks each of 5000 L water holding capacity (Tank-1: 500 individuals/m^3, Tank-2: 600 individuals/m^3, Tank-3: 700 individuals/m^3). Water quality was observed daily up to 150 days of culture period. To estimate the growth characteristics, initial and final measurements of weight and length were obtained. Survival was observed at the end of the experiment. Costs and returns were recorded to estimate economic profitability. Water quality parameters were under optimum level for fish culture and only dissolve oxygen varied significantly among the culture tanks. The highest survival rate was found 91% in tank-1. The highest mean final weight was observed 38.32 ± 1.36 g in tank-1 and the lowest mean was observed 30.60 ± 1.12 g in tank-3. Notwithstanding, food conversion ratio was the lowest 0.92 in tank-1 and the highest 1.01 in tank-3. Specific growth rate, average daily growth and health condition of fish were significantly ($p<0.05$) different among the tanks while the maximum was found in tank-1. Apart from that, production rate was observed the highest 18.21 kg/m^3 in tank-2. Lastly, the maximum benefit cost ratio (0.21) and return on investment (15.71%) were obtained. This suggests that using a stocking density of 500 individuals/m3 for *H. fossilis* in BFT aquaculture is economical.

Keywords: Biofloc, *Heteropneustes fossilis*, Shing

Introduction

Although aquaculture industry is growing fast since the 1970s, this industry has come under scrutiny for contribution to environmental degradation and pollution (FAO 2008). As a result, requirement for more ecologically sound management and culture practices remains fully necessary. Moreover, the expansion of aquaculture is also restricted due to land costs and by its strong dependence on fish feed (Browdy *et al.* 2001 and

Schryver *et al.* 2008). The ingredients of fish feed are one of the prime constituents of feed for commercial aquaculture (Naylor *et al.* 2000). Feed costs represent at least 60-70% of the total aquaculture production costs, which is predominantly due to the cost of protein component in commercial diets (Bender *et al.* 2004). Interest in closed aquaculture systems is increasing, mostly due to biosecurity, environmental and marketing advantages over conventional extensive and semi-intensive systems (Ray 2012).

The environment friendly aquaculture system called "Biofloc Technology (BFT)" is considered as an efficient alternative system since nutrients could be continuously recycled and reused. The sustainable approach of such system is based on growth of microorganism in the culture medium, benefited by the minimum or zero water exchange (Nisar *et al.* 2022). As a closed system, BFT has primordial advantage of minimizing the release of water into rivers, lakes and estuaries containing escaped animals, nutrients, organic matter and pathogens. Minimum water exchange maintains the heat and fluctuation of temperature is prevented (Crab *et al.* 2009). Another main issue raised in today's situation is water scarcity which severely ruins security and hampers the development of this sector. In aquaculture systems, Biofloc technology cultivates beneficial microbial communities. These microbes proliferate when exposed to organic stuff, such as leftover food and waste, and create thick aggregates known as Bioflocs (Nisar *et al.* 2022). By eating ammonia and other nitrogenous substances, these flocs preserve water quality and offer cultivated species a natural food source (Emerenciano *et al.* 2012). Aeration oxygenates the water and promotes floc development. Carbon supplementation also promotes floc stability and formation (Avnimelech 2015). By lowering water exchange, lessening environmental effect, and improving production efficiency through enhanced biosecurity and nutrient recycling, this technology supports sustainable aquaculture (Nisar *et al.* 2022, Avnimelech 2015). In this context in the Northern part of Bangladesh fish farming in Biofloc aquaculture system will be suitable tools to increase the fish production in this region. An essential factor in designing a Biofloc system is the species to be cultured. Biofloc systems work best with species that are able to drive some nutritional advantage from the direct consumption of floc with short life cycle fish species, which have faster growth and require low inputs, such as shing (*Heteropneustes fossilis*), magur (*Clarias batrachus*), pabda (*Ompok pabda*), silver barb (*Barbodes gonionotus*), genetically improved farmed tilapia (GIFT), etc. (Gupta 1991, Borthakur and Goswami 2007 and Shah *et al.* 2008). This study was designed for shing (*Heteropneustes fossilis*) species because it's not only recognized delicious taste and market value but is also highly esteemed from nutritional and medicinal properties of view (Azhar *et al.* 2018). So the present study aims to compare the economics of *Heteropneustes fossilis* culture in Biofloc aquaculture system.

Materials and Methods

Establishment of culture tank

Three circular culture tank were established using stainless steel pipe, food grade polyvinyl sheet and thermal insulation sheet. The main frame was built with stainless steel pipe and then the thermal insulation sheets were placed at the inner side of the frame. Finally, food grade polyvinyl sheets were set at the interior portion of the tank. An outlet was set at the side wall at the bottom of each tank. Around 45 liters'/min aeration were supplied to each tank using air stone. The diameter of each tank is 2.6 m and the height is 1.1 m. However, the water level is maintained at 5000 L in each tank.

Preparation of water and maturation of floc before stocking

At first all the tanks were filled with underground water. Aeration was continued without adding anything else for 24 hours and then continued up to the end of the experiment. Following steps in Table 1 were followed for water preparation before stocking fish seeds.

Table 1. Activities for setting up the *Heteropneustes fossilis* culture tank in biofloc

Day	Activities	
01	Water supply	
02	Addition of 50 g/m^3 CaCO$_3$ and 1 kg/m^3 raw salt	
03	Addition of 200 ml/m^3 molasses and 20 g/m^3 probiotic (Pondcare, SK+F)	Aeration and water quality monitoring
04 – 14	Waiting for the maturation of floc	
15	Stocking fish seeds (8–10 cm)	

Stocking and culture management

The Shing, *Heteropneustes fossilis* fish of 8–10 cm in length and 4.4 ± 0.26 g in body weight were stocked in April 2020 and cultured for 150 days up to September 2020. Three different levels of stocking densities were applied for the three tanks. In tank-1, tank-2 and tank-3 about 500, 600, and 700 individuals.m^{-3} were stocked, respectively. Before releasing to the tank, the fish seeds were conditioned for 30 minutes. The fish were fed three times a day initially with floating feed having 35% protein content at the rate of 5% body weight. With the increasing body weight, the feeding rate was dropped down to 2% body weight gradually and the protein content of feed was 30%. Molasses was added daily to maintain C:N ratio 15:1 in the tank water. Following equations were

applied to estimate the amount of molasses needed to maintain C:N ratio 15:1 in the tank water:

Estimating for 30% protein source: 1 kg feed contains 300 g crude protein and 100 g protein contain 16 g N. So, 300 g contain= (16✖ 3) g= 48 g N. Fish use 25 % of N and release 75% of N; So, 75% N= 36 g N. That means 1 kg feed release 36 g N in water.
To maintain C:N ratio 15:1 we need 540 g C.
Again, feed contains 50% C as being a biological substance. 1 kg feed contains= (1000✖ 0.5) g= 500 g C. Fish use 15% C and release 85% C in water. So, from 1 kg feed (500✖ 0.85) g or 425 g C will be released in water.
So, we need (540− 425) g or 115 g extra C to maintain C:N ratio 15:1. Molasses contains 24% C. That is 1 kg molasses contains 240 g C. For 115 g C, we need $\dfrac{115 \times 1000}{240}$ g $\approx$ 479 g molasses.

Water quality parameters
Water quality parameters were observed daily during the culture period to estimate the environmental condition of the tanks. Air temperature (°C) and water temperature (°C) were observed using digital thermometer. Water pH and total dissolve solids (mg/L) were measured using digital meter (Hanna). Dissolve oxygen (mg/L) was observed using DO meter (Lutron, DO-5509). Ammonia (mg/L) was measured using Hach kit and floc density (ml/L) was observed using Imhoff cone.

Growth parameters
Initial weight (g), final weight (g), initial length (cm) and survival were recorded to estimate live weight gain (g), specific growth rate (% day^{-1}), average daily growth (g day^{-1}), health condition (g cm^{-1}), survival rate (%), and production rate (kg m^{-3}). Following calculations were applied to estimate these parameters:

SGR (% day^{-1})	:	$\dfrac{\ln(\text{Finalweight}) - \ln(\text{Initialweight})}{\text{Cultureperiod(Days)}} \times 100$
ADG (g day^{-1})	:	$\dfrac{\text{Finalweight} - \text{Initialweight}}{\text{Cultureperiod(Days)}}$
Health condition (g cm^{-1})	:	Final body weight/Total length
FCR	:	Dry feed fed/ Live weight gain
Production (kg m^{-3})	:	Total weight of the harvested fish/Volume of water.

Economic analysis

Gross return, net profit, return on investment, and benefit cost ratio were estimated from the data represented in Tables 2–5 to find the economic viability of *Heteropneustes fossilis* culture in biofloc in Northern region of Bangladesh.

Table 2. Fixed cost and depreciation cost of initial setup for *Heteropneustes fossilis* culture in the three Biofloc tanks

Sl No.	Description of Items	Unit Price	Quantity	Total price (Tk)
1	Circular tank of polyvinyl sheet	8,000/-	3 pcs	24,000/-
2	Frame for circular tank	12,000/-	3 pcs	36,000/-
3	Thermal insulation sheet	2,500/-	3 pcs	7,500/-
4	Air pump	14,800/-	1 pc	14,800/-
5	Air stone	95/-	21 pcs	1,995/-
6	Air pipe	3/-	300 foot	900/-
7	TDS + Temperature meter	1,500/-	1 pc	1,500/-
8	pH meter	1,500/-	1 pc	1,500/-
9	DO meter	4,800/-	1 pc	4,800/-
10	Imhoff cone	1,000/-	1 pc	1,000/-
11	IPS (1200 watt)	20,000/-	1 pc	20,000/-
12	Contingencies	-	-	2,000/-
	Total			**115,995/-**
	Fixed cost per tank			**38,665/-**
	Depreciation cost per tank in each culture period (10 % of fixed cost)			**3,867/-**

*Cost per tank = Total cost/3; Longevity of the tank is considered 5 years. In each year, two culture cycle can be executed

Table 3. Operation cost A (Excluding feed and molasses) of *Heteropneustes fossilis* culture in the three Biofloc tanks

Sl No.	Description of Items	Unit Price	Quantity	Total price (Tk)
1	Shing fish fry	2/-	9000 indi.	18,000/-
2	Probiotic (Pondcare, SK+F)	495/-	6 pc	2,970/-
3	Raw Salt	12/-	15 kg	300/-
4	Electricity cost (120 Unit×5 months)	4.18/-	600 Unit	2,508/-
5	Ammonia test kit	2,000/-	1 pc	2,000/-
6	Miscellaneous (Scoop net, Feed storage, Bucket etc.)	-		4,000/-
	Total			**29,778/-**
	Operation cost A per tank			**9,926/-**

*Operation cost A per tank = Total operation cost A/3

Table 4. Operation cost B (Only feed and molasses) of *Heteropneustes fossilis* culture in the three Biofloc tanks

Tank	Description of Items	Unit Price (Taka)	Quantity (kg)	Total Price (Taka)
01	Feed	65/-	80	5,200/-
	Molasses	30/-	38	1,140/-
	Sub Total			6,340/-
02	Feed	65/-	91	5,915/-
	Molasses	30/-	43.5	1,305/-
	Sub Total			7,220/-
03	Feed	65/-	89	5,785/-
	Molasses	30/-	42.5	1,275/-
	Sub Total			7,060/-

Table 5. Income from *Heteropneustes fossilis* fish selling in the local market of Northern region of Bangladesh

Tank	Grade	TP (Kg)	Unit Price (Taka)	Sub Total Price (Taka)	Total Price (Taka)
01	A	24.50	300/-	7,350/-	24,447/-
	B	47.80	280/-	13,384/-	
	C	14.85	250/-	3,713/-	
02	A	17.75	300/-	5,325/-	25,009/-
	B	45.30	280/-	12,684/-	
	C	28.00	250/-	7,000/-	
03	A	5.5	300/-	1,650/-	23,689/-
	B	48.80	280/-	13,664/-	
	C	33.5	250/-	8,375/-	

*Grade A: 22-26 individual's kg-1; Grade B: 27-30 individual's kg-1; Grade C: 31-34 individual's kg-1; TP: Total production.

Following calculations were used to estimate net profit and payback period:

Net profit (per cycle)= Gross return per cycle − [(Depreciation cost + Operational cost) (A+B)]

Where, Depreciation cost is the 10% of fixed cost.

Return on Investment= (Net profit $\times 2$) /Initial Investment) $\times$ 100 %; as each year consists two culture cycle.

Where, Initial investment= Fixed cost + Operational cost (A+B)

Benefit Cost Ratio= Net Profit per cycle/[Depreciation cost + Operational Cost (A+B)]

Statistical analysis

Mean and standard error of mean were calculated using MS excel. One-way multivariate analysis of variance was executed to find the significant differences among the different stocking densities. Normality and heteroscedasticity of the data were tested prior to the analysis. When the assumptions were met, Tukey's post-hoc test was performed to categorize the tanks with different stocking densities of fish. Level of significance was set at 0.05. Statistical analysis was performed using SPSS (version 25.0, IBM).

Results

Water quality parameters

Water quality parameters were mostly similar in all the three tanks (Table 6). Only dissolve oxygen varied significantly ($p<0.05$) among the culture tanks. Water temperature varied from 30.3 – 25.9 °C, water pH varied from 7.7 – 7.2, dissolve oxygen varied from 5.9 – 4.1 mg/L, ammonia varied from 0.07 – 0.03 mg/L, total dissolve solids varied from 1750 – 1516 mg/L, and floc density varied from 27 – 14 ml/L across the three culture tanks.

Table 6. Water quality parameters of *Heteropneustes fossilis* culture tanks in Biofloc system. Values are mean with standard error (SE = σ/√n). Values with different letters within each row represents significant ($p<0.05$) difference

Parameters	Tanks		
	T_1	T_2	T_3
Air temperature (°C)	30.4 ± 0.10	30.4 ± 0.15	30.4 ± 0.14
Water temperature (°C)	28.3 ± 0.12	28.1 ± 0.16	28.1 ± 0.18
Water pH	7.5 ± 0.02	7.5 ± 0.02	7.4 ± 0.02
DO (mg/L)	5.4 ± 0.04[a]	5.1 ± 0.03[b]	4.5 ± 0.03[c]
Ammonia (mg/L)	0.06 ± 0.001	0.05 ± 0.001	0.05 ± 0.001
TDS (mg/L)	1668 ± 6.70	1622 ± 3.78	1588 ± 5.85
Floc density (ml/L)	22± 0.41	20± 0.49	21±0.32

Growth performance

The highest weight gain and survival rate were observed in tank-1 whereas the lowest were recorded in the tank-3 (Table 7). Notwithstanding, the production rate was the highest in tank-2. However, food conversion ratio was the lowest in tank-1 and the highest in tank-3. On the other hand, statistical analysis showed that the variation of specific growth rate, average daily growth and health condition were significant ($p<0.05$)

among the three culture tanks (Fig. 1). All of these three parameters were recorded the highest in tank-1 and the lowest in tank-3.

Table 7. Growth parameters of *Heteropneustes fossilis* culture tanks in Biofloc system. Values are mean with standard error (SE = σ/√n)

Growth parameters	Tanks		
	T_1	T_2	T_3
Initial weight (g)	4.40 ± 1.70	4.40 ± 1.70	4.40 ± 1.70
Final weight (g)	38.32 ± 1.36	35.71 ± 1.47	30.60 ± 1.12
FCR	0.92	1.00	1.01
Survival (%)	91	85	82
Production (kg m^{-3})	17.43	18.21	17.56
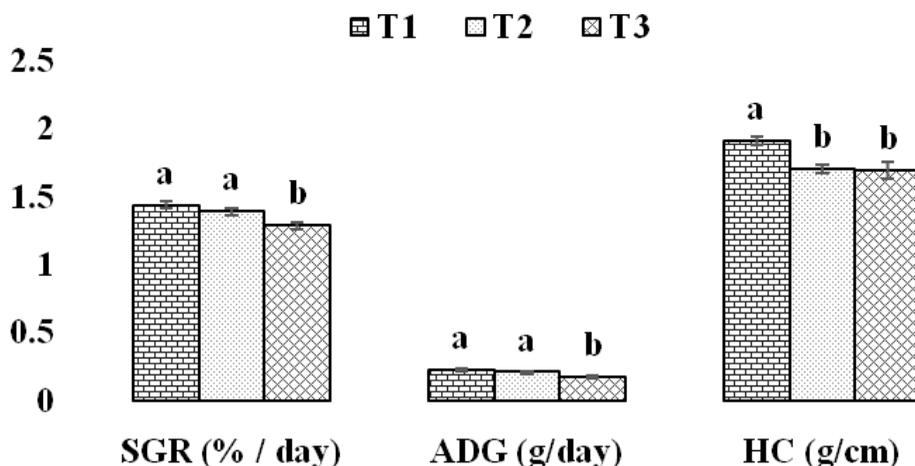
Fig. 1. Specific growth rate, SGR(%/day), average daily growth, ADG(g/day), and health condition, HC (g/cm) of *Heteropneustes fossilis*, cultured in Biofloc. Values are mean with standard error (SE = σ/√n) bars. Values with different letters represents significant difference ($p<0.05$) within each category.

Economic viability
Economic viability of *Heteropneustes fossilis* culture under different stocking density is represented with the net profitability, return on investment and benefit cost ratio (Table 8). The highest net profit, return on investment and benefit cost ratio was observed in the tank-1 and the lowest was observed in the tank-3.

Table 8. Economic return from *Heteropneustes fossilis* culture in Biofloc system

Tank	Total Cost (Taka)	Gross Return (Taka)	Net Profit (Taka)	Return on Investment (%, Annual)	Benefit Cost Ratio
1	20,133/-	24,447/-	4,314/-	15.71	0.21
2	21,013/-	25,009 /-	3,996/-	14.32	0.19
3	20,853/-	23,689/-	2,836/-	10.19	0.14

Discussion

Water quality

Most of the water quality parameters were in optimum level for fish culture and did not varied significantly. BFT is a sustainable and environmentally-friendly method of aquaculture that controls water quality and harmful pathogens along with providing value-added production of microbial protein feed for the aquatic farm system (Ray 2017)**.** However, dissolve oxygen level was significantly different among the tanks and the maximum DO level was observed in tank-1 that is for the lowest stocking density, and the minimum DO level was observed in tank-3 that is for the highest stocking density. Notwithstanding, ammonia level did not vary likewise the variation of dissolve oxygen. The promoted nitrogen uptake by bacterial growth decreases the ammonium concentration more rapidly than nitrification (Hargreaves 2006). Therefore, bacterial action was observed more effective in tank-2 and tank-3 resulting in efficient conversion of ammonium even with the higher stocking density and comparatively lower oxygen level. Usually floc maturation prior to the stocking of fish greatly affects the bacterial action on ammonia conversion (Avnimelech 2015). So, the results also indicate that the floc in tank-2 and tank-3 were matured at optimum level for maintaining the functional system of Biofloc technology.

Growth parameters

Initial and final weight of fish represents that the live weight gain of fish was the highest in tank-1 with the lowest stocking density. A study by Shamsuddin et al. (2022) reported that in Biofloc system lower fish stocking density has a higher opportunity of feed and space; and a lower chance of stress and physical injury; thus, lower stocking densities has better growth performance. This study also agreed with that finding. Inversely, the food conversion ratio was the lowest in tank-1 with the lowest stocking density. Addition of carbohydrates to culture environment stimulates heterotrophic bacterial growth and nitrogen uptake through the production of microbial proteins takes place (Avnimelech 1999). If carbon and nitrogen are well balanced in the solution, ammonium in addition to organic nitrogenous waste will be converted into bacterial biomass

(Schneider *et al*. 2005). Although the highest production rate was observed in tank-2 but it happened due to the higher stocking density than tank-1, higher survival rate than tank-3, and lower FCR than tank-3. Survival rate was observed to be affected by spatial and food competition with the increasing stocking density. However, the survival rate of *Heteropneustes fossilis* (91–82%) in treated tanks was slightly lower than the results (92.22–96.67%) of *Clarias gariepinus* obtained by Chen et al. (2020). Apart from that, SGR and ADG did not varied significantly between tank-1 and tank-2 but both are significantly different from tank-3 and tank-3 showed the lowest SGR and ADG. On the other hand, health condition of fish from tank-1 was the best among the three tanks while HC of fish from tank-2 and tank-3 was mostly homogenous. Fish and shrimp are grown in an intensive way (minimum of 300 g of biomass per square meter (Emerenciano *et al*. 2012) with zero or minimum water exchange. The consumption of Biofloc by shrimp or fish has already demonstrated improvement of growth rate and decrease of FCR (Avnimelech 2015)**.**

Economic viability

Net profit from all of the tanks showed that all the farms will be viable as the revenue exceeds the production cost (Cheremisinoff 1995). However, return on investment and benefit cost ratio decreased with the increasing stocking density. Nowadays, feed cost is increasing thus the increased FCR resulted in higher production cost. In different fish culture systems, survival and FCR are important growth indices representing gains or losses in the aqua business (Sgnaulin *et al.* 2018). Nonetheless, the microbial protein consumption can decrease costs in feed (Avnimelech 2015)**.** Based on the returns from all the three tank it would be wise to apply 500 individuals/m^3 stocking density for *Heteropneustes fossilis* culture in biofloc to ensure the enterprise economically viable.

This study found *Heteropneustes fossilis* culture in Biofloc system economically feasible for Northern region of Bangladesh. Besides, the growth parameters and the return showed that the maximum production and return from the minimum production cost will be ensured from the 500 individuals/m^3 stocking density in Biofloc system. However, the price of the fish may vary in other region of Bangladesh which may contradict this results. Contrarily, the production cost may also be reduced by using the handmade feed rather than commercial feed with high cost. Further study should be carried out on the improvement of the quality and health condition of the product that may bring more benefits.

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Present status of the fish seed producing hatcheries in the Jashore region, Bangladesh

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Abstract

The study was carried out on 34 fish hatcheries during March to august, 2023 to assess the present status of fish fry, hatchlings production and trade at Chanchra under Jashore district. Data were collected through questionnaire interviews by using personal interviews and participatory rural appraisal (PRA) tools. Fifty percent (50%) of the hatcheries have own source of broods, 30% and 20% broods from other ponds and natural sources (Halda and Jamuna River) respectively. Carp hatchlings that produced in hatcheries were about 15 species including Indian major carps, exotic carps like Silver carp, Grass carp, Black Carp, Bighead carp, Common carp, Mirror carp and other species like Pangus, Punti, Thai punti, Koi and Tilapia. Hatchlings production of Indian major carps were 34,720 kg; Exotic carps were 31,754 kg; and other species were 3,746 kg, respectively in 2023. Fry production of Indian major carps were 1067,150 kg; monosex tilapia was 16,200 kg and other species were 1,714 kg. It was estimated that 70,220 kg hatchlings were produced from 34 hatcheries and 1065,064 kg fry were produced from 34 nurseries in the year 2023. The price of the hatchlings of different species varies from 2,000-30,000 taka/kg year round. The average price of fish seed was maximum in monsoon and minimum in winter. It was observed that the marketing channel consisted of brood fish, hatchlings production, nursery owners, fry production, wholesaler, retailer, and farmer. Fry were distributed from Jashore to south-west region (46%), south-east region (19%), north-west region (18%) and only 17% in north-west region. Only 33% of the hatchery operators attained training. In the present study 90% of the hatcheries do not get any lab facilities and 11% hatcheries were stopped their production due to several constraints.
Keywords: Hatchlings, Fry and fingerling, Transportation and Marketing

Introduction

Good quality fish seed is essential for contributing the up growing trend in aquaculture. While a minor amount is harvested from rivers, the primary source of fish seed in Bangladesh is spawn generated in public and private hatcheries. Fish spawn is produced in 984 hatcheries in Bangladesh, weighing about 627,586 kg (DoF 2023). For sustained aquaculture, it's crucial to make sure there is a constant supply of high-quality fish fries. The poor breeding response, coupled with a relatively shorter spawning season, results

in an inadequate production of hatchery reared seed, which often fails to cover the entire needs of the farmers (Rashid et al. 2022). Fish hatcheries and nurseries initially developed in four major clusters in Mymensingh, Jashore, Bogura, and Cumilla regions, close to government fisheries research stations or where nursing of wild riverine seed was a traditional activity. They have subsequently become established throughout the country as technicians from these centers have been employed to set up new operations or have established their own. In the 1980s, hatchlings were produced and supplied from hatcheries in Jashore in Southwest Bangladesh over 100 miles away from the Northwest region (Haque 2007). During 1995, fish seed market was introduced in Chanchra area, Jashore with a large number of nursery ponds. (Belton *et al.* 2011). Since then, the number of fish hatchery has increased uninterruptedly reaching over a thousand in 2010 to fulfill the ever-increasing demand of the fin fish seeds for aquaculture industry of Bangladesh. Nowadays, over 33 hatcheries are running their business in Chanchra area in Jashore. In the fiscal year 2022-23, the district surpassed all expectations, producing a staggering 241,107 tons of fish and consistently produced a surplus of 497,795 metric tons of fish over the past three years, surpassing local demand.

The fish marketing system in Bangladesh is traditional, complex, and less competitive but plays a vital role in connecting the fish producers, and consumers, thus contributing significantly in 'value add' process of the fish which otherwise would have been unused or underused and consequently in the earnings of the fisher folk (Chowdhury 2004). In fish seed marketing system of Chanchra, Jashore, a number of intermediaries are involved actively for selling fish seeds. The market chain from seed producers to fish farmers passes through a number of intermediaries: local fish seed retailers, agents, seed wholesalers and retailers. A pre order for fish fry should be made for seed trading. Both traders and retailers arranged transportation for seed. The hatcheries with a reputation for producing better quality seed experience very high customer demand and are able to obtain a premium for the spawn they produce, suggesting that economic incentives for good hatchery management do exist. However, the marketing channel and problems associated with marketing of fish seed in Jashore region has not been properly quantified. Most of the hatchery owners did not follow aquaculture code of conduct, breeding protocols, brood stock and hatchery management technology. The present study was undertaken to justify the present status of fish seed producing hatcheries of Jashore region.

Materials and Methods

Study area and data collection

Fish hatchery production is dominant in the south-western zone. The south-western zone has a lower temperature and rainfall range; however, the Jashore district, which harbors numerous hatcheries experiences the highest temperature. The present study was conducted on Chanchra under Sader upazilla of Jashore district where production season of fish seed generally started from 1st week of April and ends up by 1st week of September every year. However, the survey was carried out for six months from April to August 2023.

A total of 34 hatcheries were randomly selected for the collection of data. Prior to collect data a structured questionnaire was prepared. The questionnaire was prepared adhering to the objective of the study. Surveys were conducted using the formulated questionnaire in respective hatcheries. Fry production practices including application of different inputs by the hatchery technicians were observed closely and recorded. Data on total fry production in different hatcheries including production cost and return achieved were collected. FGD (Focus Group Discussion), PRA (participatory Rural Appraisal), RRA (Rapid Rural Appraisal), Collection of secondary data were used to collect data**.** The secondary data were collected from different website of Journals; District Fisheries Office, Jashore; Upazilla Fisheries Office, Jashore etc.

Data processing and analysis

The collected data were scrutinized and summarized carefully before the actual tabulation. Some of the collected data were in local units due to respondent's familiarity with those units. These data of local units were converted into international units before transferring to the computer. The qualitative data were categorized and analysis mainly based on descriptive statistical analysis by MS excel and SPSS (Statistical Package for Social science) version 23. All the collected data were processed and analyzed to extract the findings of the study following careful accumulation.

Results

In Jashore region three types of hatcheries were observed which are Carp, (Carp +Catfish) and (Carp+Catfish+Tilapia). Carp contributed the most part (70%) where minimum contribution from carp, catfish and tilapia hatchery (Fig. 1).
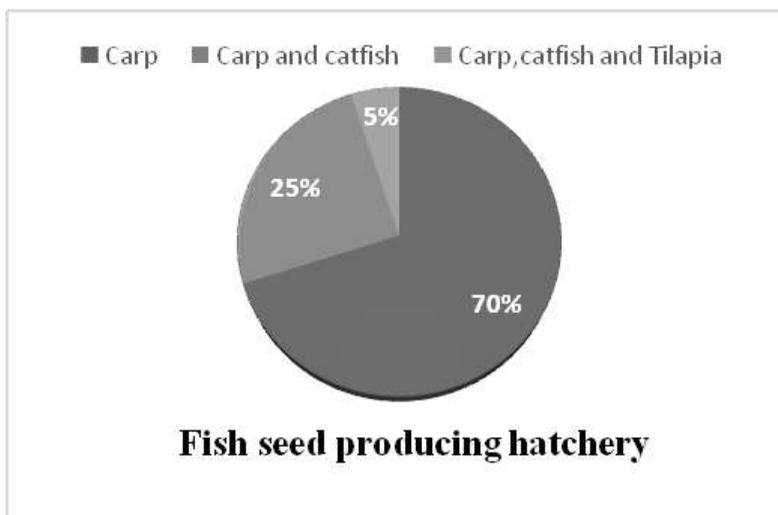
Fig. 1. Percentage of fish seed producing hatchery in Jashore region.

Source of brood

After observations three types of brood sources were found. Fifty percent (50%) of the hatcheries have own source of broods, thirty percent (30%) use broods from other ponds and twenty percent (20%) of hatcheries use broods from natural sources (Fig. 2).
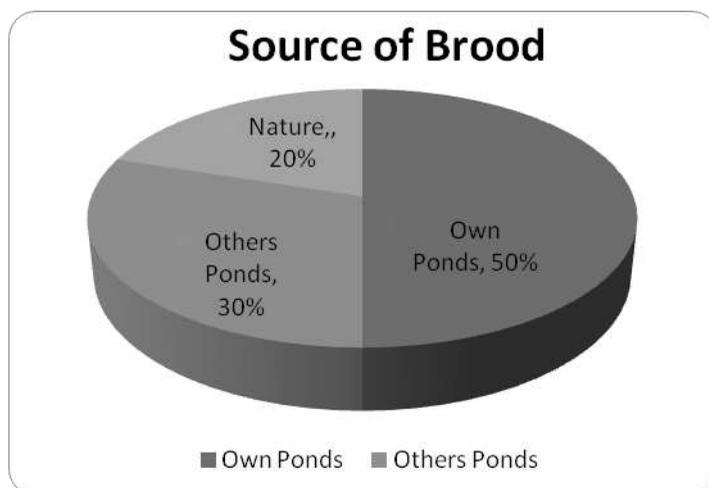

Fig. 2. Percentage of brood sources for hatcheries in study region.

Hatchling production

Hatcheries of Jashore district produce three types of hatchlings mainly Indian major carps (IMCs), exotic, other species. Among the produced hatchlings IMCs produced by 48% hatcheries, exotic hatchlings by 43% of hatcheries and only 9% hatchery produce other species (Fig. 3).
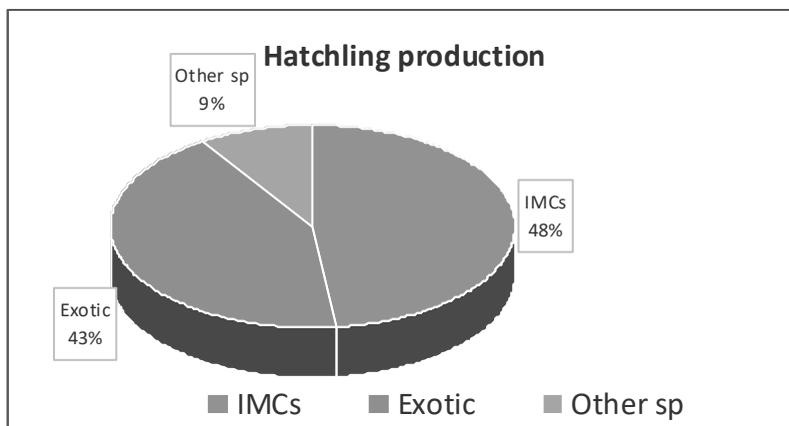
Fig. 3. Percentage of hatchling production in hatcheries in the research area.

Hatcheries produced hatchlings are distributed mostly all over Bangladesh from Jashore where 46% of hatchlings are distributed in south-west region, 19% distributed in south-east region, 18% in north-west region and only 17% in north-west region (Fig. 4).
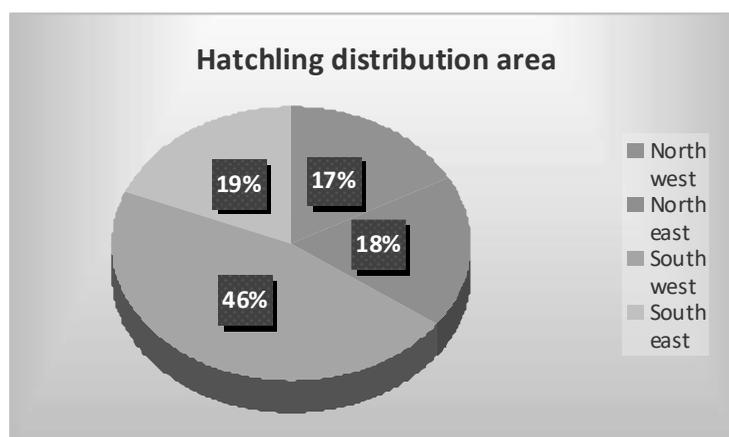
Fig. 4. Percentage of hatchling distribution from hatcheries in the research area.

Price of hatchlings

The price of fry in Jashore was being fixed by the hatchery owners. However, the price variation was seen due to seasonal variation, species and demand. The average price of fish seed was maximum in monsoon and minimum in winter. Average prices of hatchlings of different species are shown in Table 1.

Table 1. Price of hatchlings of different carp species

Species (Common name)	Scientific name	Price (Taka/kg)
Rui	*Labeo rohita*	3000-5000
Catla	*Catla catla*	2000-6000
Mrigal	*Cirrhina cirrhosus*	1500-4000
Silver carp	*Hypophthalmichtys molitrix*	2000
Grass carp	*Ctenopharyngodon idella*	4000
Mirror carp	*Cyprinus carpio var. specularis*	2500-3000
Common carp	*Cyprinus carpio var. flavipinnis*	2500-3000
Kalbaus	*Labeo calbasu*	3000-4000
Thai punti	*Puntius gonionotus*	1500-2000
Pangus	*Pangasius pangasius*	3000-3500
Tilapia	*Oreochromis mossambicus*	2 /piece
Black carp	*Mylopharyngodon piceus*	12-15/piece

In case of lab facilities almost 90% of the hatcheries did not get any lab facilities where only 10% got lab facilities like having microscope, soil test kits and portable testing facilities (temperature, pH & ammonia) (Fig. 5).
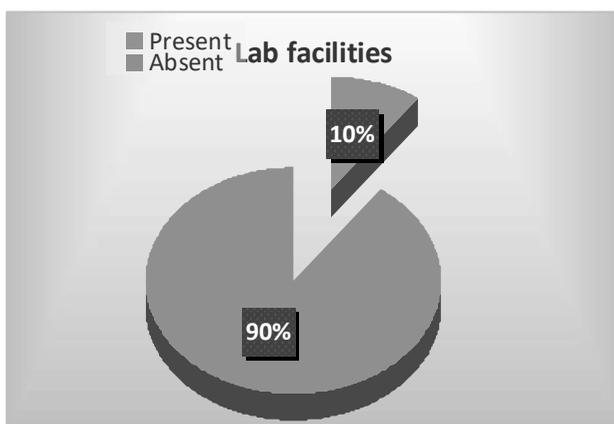

Fig. 5. Percentage of lab facilities of hatcheries in the research area.

Training

Training is very important to run a work properly and scientifically. In the study area, it was noticed that only 33% of the hatchery operators attained training and the other had no training knowledge.
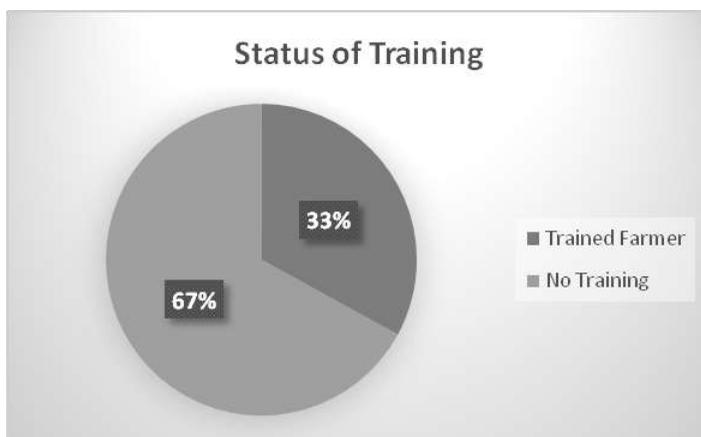

Fig. 6. Status of training of the farmers.

About 38 hatcheries were surveyed during the study period. Among them 34 hatcheries (89%) were running and 4 hatcheries (11%) were stopped their production due to several constraints like diseases problem, lack of capital and credit facilities, high lease value of pond, shortage of underground water, high rate of Electricity, high rate of production inputs, high temperature, intense market competition, lack of policy support, presence of iron in water more than normal etc. (Fig. 6).

Among the surveyed 34 hatcheries, it was found that 21 hatcheries had involvement with skilled technicians but 13 hatcheries had no involvement with skilled technician rather they were engaged themselves in hatchery activities.

Marketing channel of fish fry

Marketing of hatcheries in Jashore district included fish hatchery, nursery, wholesalers, fry traders and fish farmers. Fish fry and fingerling marketing channel was observed during the study period is shown in diagram (Fig. 7). We found that there were two ways of seed production in hatcheries of Chanchra i.e collecting wild egg of fish from river, estuary, hoar and other was occurring induced breeding between well qualified brood-stock of that particular species like *Cirrhinus cirrhosus, Labeo calbasu, Labeo rohita, Labeo bata, Catla catla, Aristichthys nobilis, Ctenopharyngodon idellus, Cyprinus carpio,*

Cyprinus carpio, Mylopharyngodon piceus, Hypophthalmichthys molitrix, Pangasianodon hypophthalmus, Mystus tengara, Puntius gonionotus, Anabas testudineus and Oreochromis mossambicus according to the present status during survey. Everyday more than 350 fry traders come here and carry the fry by means of van, pick-up van, bus, truck, train, nosimon etc. The seeds were transported broadly from Chanchra to Shatkhira, Jhenidah, Meherpur, Kustia, Dhaka, Khulna, Magura, Norail, Razbari, Faridpur, Madaripur, Gopalgang, Manikgang & Bagherhatvai various size of truck containing seed-full oxygenated bag.
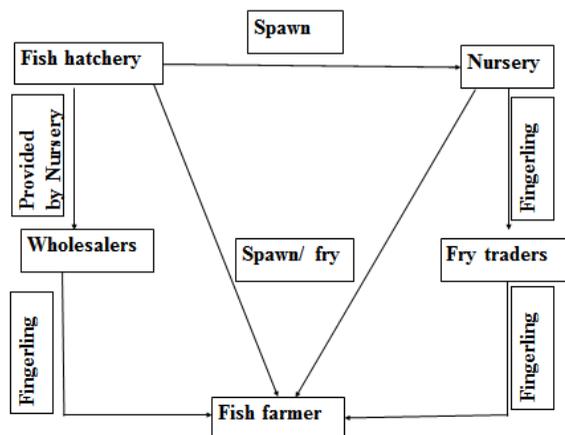

Fig. 7. Fish fry and fingerling marketing channel in Jashore district.

Packaging and transportation
About 250-300g of fry was packed in oxygenated polythene bag of 99.44 cm length and 45.72 cm breadth with two third of water and rest of oxygen.

Discussion

Present study shows among the 34 hatcheries twenty four hatcheries produced only carp, nine hatcheries produced carp with catfish and one hatchery produced carp, catfish and tilapia similar carps, similar types of findings were found by the study of Asif *et al.* (2014), Asif *et al.* (2015), Rahman *et al.* (2015), Sharif and Asif (2015), Hossain *et al.* (2016), Shabuj *et al.* (2016a), Islam *et al.* (2016), Ali *et al.* (2016a) and Ali *et al.* (2016b). It was found that 50% hatchery owners collected brood from their own ponds, 30% hatchery owners collected brood from the other hatcheries and 20% hatchery owners collected broods from the natural sources like the Padma and the Halda River which is linked with Samad *et al.* (2013), Hossain *et al.* (2016), Shabuj *et al.* (2016a),

Islam *et al.* (2016), Islam *et al.* (2017) and Ali *et al.* (2018) study. Present study shows hatchlings production of Indian major carps were 34,720 kg, exotic carps were 31,754 kg, and other species were 3,746 kg respectively in 2022 at Jessore. Rahman (2007) reported that in Jashore 42,11.5 kg hatchling were produced in 85 hatcheries in this area of which 80% were reared in the same area. The findings is relevant with present study. Again, Rahman (2007) reported that a total 1,621 lacs of 1-4" sized fry was produced during the study period. Among them Indian major carp 864.3 lacs (53.32%), Chinese carp 621.9 lacs (38.36%), Thai shar punti and Pangus 134.8 lacs (8.32%). Present study shows that, among the produced hatchlings IMCs produced by 48% hatcheries, exotic hatchlings by 43% of hatcheries and only 9% hatchery produce other species. The distribution area of present study was more or less similar with Asif *et al*. (2014) study. In the present study, hatchling was distributed from Jashore to south-west region 46%, south-east region 19%, north-west region 18% and only 17% in north-west region. Sharif *et al.* (2015) stated that fry production of Indian major carps was 399,596 kg, exotic carps were 391,272 kg, and other species were 41,625 kg in 2013 at Jashore. Previous study and present study is more or less similar at the area of fry production. With the existing facilities, the hatcheries surveyed produced quantity of 70,220 kg hatchlings and 1065,064 kg fry were produced from 34 nurseries at Jashore in the year 2023. The price of the hatchlings of different species varies through the year round. It varies from 2,000-30,000 taka. The price of hatchling is more or less similar to the study of Rahman (2007). He reported that average price of per kg hatchling were Indian major carps- Tk.1200 to 1800, Chinese carp Tk. 1200 to 1600 and Thai sharpunti and Pangus- Tk. 900 to 1200. Asif *et al.* (2014) stated that training is very much essential to run a work properly and scientifically. In Jessore region it was observed that only 30% of the fish fry and fingerling traders attained training and the others had no training knowledge. In the present study, it was observed that only 33% of the hatchery operators attained training and the rest had no training knowledge. This is more or less similar with the present study.

The fry produced from the hatcheries in the area were distributed in most parts of the country. Fry traders used to come from Khulna, Satkhira, Bagarhat, Barishal, Jhalukati, Comilla, Chandpur, Chuadanga, Dhaka, Borguna, Faridpur, Madaripur, Kushtia, Magura, Naraial, Pabna, Bogura, Rangpur, Nator, Mymensingh and other districts of Bangladesh. Sometimes fry and fingerling exported from Bangladesh to Assam, West Bengal and adjacent part of India, (Asif *et al.* 2014). In the survey study area, it has been found that most of the hatchery owners used oxygenated drum for long distance fry were transported by pick up or truck (large quantity) and locally for small fry traders, they were transported cloth bag by van, rickshaw. Ali *et al.* (2018) reported that transportation is essential for live product like fish fry. Oxygenated bag is widely used

for transportation of hatchlings. An oxygenated bag is containing 250-300 g hatchlings for the best use within 24 hrs. For carrying fry or fingerlings they use pot or plastic barrel. Traders are aware of the requirements of oxygen for fry, so they continuously agitate the water in the pot during transportation. The fry was found to transport by means of bus, truck, train, pick-up, auto rickshaw, van etc. In local markets, the fry is transported by van, metallic pot, public bus and some cases by truck. But train, truck or pick-up are found to be the main means of transportation of fry to the far way districts. Lewis (1996) reported that *Labeo rohita* fry are relatively sensitive to the stress of transport; as a result, there is a high mortality among transported fry. Asif *et al.* (2014) studied that aluminum bowl are used to 49% transportation of fry and fingerling, 32% used to plastic drum, 7% is used oxygenating poly bag and rest 12% are transported by oxygenating drum. Currently to address the mortality problem almost 100% transportation is conducted by oxygenated poly bag. Quality seed production is the prerequisite for sustainable aquaculture. In Bangladesh, production of fish seed was not a problem but the crucial factor was to maintain its quality. Over the last two decades Bangladesh became self-sufficient to produce and distribute fry to the users but the quality of fish seed had been deteriorating day by day. Inbreeding, inter specific hybridization, negative selection of broods, improper brood stock management were common phenomena in hatcheries especially in the private hatcheries. These factors resulted in low growth rate, high mortality, deformities, less fecundity and so on.

Aquaculture principally relies on the availability of fry and fingerlings from hatcheries. Based on the market demand, the business has been well flourished and a lot of people were involved in this trade and improved their socio economic condition, but some problems were evident in the present study. For this reason, hatchery owners faced economic loss to some extent. Unless decentralized fish seed production includes appropriate breeding strategies to maintain the genetic quality of brood stock, the performance of the production stocks will decline. If awareness can be raised through extension, technical support, different training to the farmers, assistance programs, improvement in agri-mechanization, proper solution to the inbreeding problems and market security, there is no doubt Jashore will be self-sufficient that leads to food and economic security in the country. Furthermore, Good Aquaculture Practices (GAP) in the aquaculture industry could provide a potential solution to the many levels of uncertainty and reassure buyers, retailers, and civil society regarding these concerns.

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Indoor and outdoor culture of live feeds and comparison of growth performance

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Abstract

Live feed is the basic food source and nutrient security for successful seed production of any commercially important aquaculture species of fishes, mollusks and crustaceans. In the present study, indoor and outdoor culture of three live feed (Microalgae) species *Nannochloropsis* sp., *Nannochlorum* sp. and *Tetraselmis* sp*.* were conducted to scale up the production in prepared F_2 media (stock solution prepared by mixing chemicals) and to compare their growth performance in indoor and outdoor culture condition. In indoor culture condition the growth pattern obtained for three microalgae were *Tetraselmis* sp*.> Nannochlorum* sp*.> Nannochloropsis* sp*.* On the other hand, in outdoor culture *Nannochloropsis* sp. showed highest growth $3.03 \times 10^6 \pm 1.40$. Significant difference was found in both culture condition in case of *Nannochlorum* sp. and *Tetraselmis* sp., except *Nannochloropsis* sp. Environmental parameters for optimal growth of different microalgae species are different and the growth pattern is also species specific. So, the reasons behind the differences in growth performance of the studied species in same culture condition might be the effects of environmental parameters and different growth pattern. On the other hand, the outdoor culture is greatly susceptible to environmental fluctuations and contamination than the indoor controlled environment, this might be the reason of lower growth performance in outdoor than indoor.

Keywords: *Nannochloropsis* sp., Microalgae, Live feed

Introduction

Microalgae or microphytes are single-celled autotrophic microscopic phytoplankton naturally found in both freshwater and marine environments. In aquaculture, microalgal production is primarily conducted to provide live feed for mollusk culture, as well as for larval rearing of shrimp and fish. The significance of plankton in fishery growth is crucial for their distribution, both qualitatively and quantitatively (Haque *et al.* 2015). Cultivation of larval stages of various aquaculture species is highly dependent on live feed (Maehre *et al.* 2013, Eryalcin 2018). Microalgae are also an inevitable source of nutrition for most of the shrimp larvae in captive rearing (Pulz and Gross 2004). The practice of rearing marine finfish larvae in the presence of microalgae is typically

associated with higher growth rates than when larvae are reared in clear water (Conceicao *et al.* 2010). Most microalgae are rich sources of essential fatty acids; vitamins such as B_{12}, B_6, B_1, biotin, riboflavin, nicotinic acid, pantothenate, C, E and A; chlorophyll 'a' & 'b' and carotenoids. These plankton play a vital role in aquaculture to meet the nutritional requirements of the larvae as well as for bio-encapsulation (CMFRI 2009). The *Nannochloropsis* sp., *Nannochlorum* sp. and *Tetraselmis* sp. are rich with relatively high content of essential fatty acids in comparison to other marine algae (Ohse *et al.* 2015). Used in conjunction with Nannochloropsis to nourish brine shrimp, oysters, clams, mussels, and scallops, Tetraselmis sp. are exceptional larval shrimp food and contain natural amino acids that stimulate nutrition in marine organisms (Hemaiswarya *et al.* 2011). As the production of microalgae is considered expensive (Mahata *et al.* 2022), several approaches have been attempted to replace microalgae in the hatchery with artificial diets but microalgae still cannot be replaced fully with other types of feed, such as microbound and other enrichment diets (Sanchez *et al.* 2012, Ma and Qin 2012). However, live feeds are needed to be enriched to enhance the qualitative and quantitative nutrients, especially the essential fatty acids of the HUFA's. Therefore, scaling up of species specific and cost effective culture and production technique of live feed has become a prime requirement for the last few years. So, the objective of this study was to scale up the production of three live feed species in prepared F_2 media under indoor and outdoor culture condition and to compare their growth performance in the two culture condition.

Methodology

Study Location and Duration
The research was conducted in the live feed laboratory of Bangladesh Fisheries Research Institute (BFRI), Brackishwater station, Paikgacha, Khulna from April 2017 to September 2018.

Experimental design and culture media for live feed
F_2 media is a stock solution prepared by mixing the chemicals according to Guillard (1975). Experiments were implemented for 15 days and the experiments were repeated for thrice. Performance of live feed were evaluated from the cell density. 0.5 ml/L of F_2 media were added in filtered seawater in the culture vessel. Inoculum density was 5-10% of culture volume in both indoor and outdoor culture. Light intensity was maintained from 1500 to 2000 Lux for 24 hours with a constant temperature of 20-25°C in the indoor controlled condition. For outdoor culture, a 60L white container with seawater was used under day-night photoperiod condition. Culture of live feed (microalgae) was performed under the following experimental condition mentioned in Table 1.

Table 1. Experimental design for culture of live feed (Microalgae)

Treat-ment	Replication	Species	Culture condition	Culture vessel	Inoculum density/ volume	Media
T_1		*Nannochloropsis* sp.				
T_2		*Nannochlorum* sp.	Indoor	2L conical flask		
T_3	3	*Tetraselmis* sp.			5-10% of culture volume	F_2 medium
T_1		*Nannochloropsis* sp.				
T_2		*Nannochlorum* sp.	Outdoor	60 L white container		
T_3		*Tetraselmis* sp.				

F_2 media = Total 5 ml of A, B, C.

Whereas,

A = [75 gm Sodium Nitrate+5 gm of Sodium Phosphate]

B = [(180 gm Manganese Chloride + 22 gm Zinc Sulphate+10 gm Copper Sulphate+10 gm Cobalt Chloride+ 6gmSodium Molybdate)= 1ml+4.36 gm EDTA+3.15 gm Ferric Chloride]

C = [20gmThiamine Hydrochloride+100gmD-Biotine+100gm Cianocobalamine]

Determination of Cell Density

Microalgae growth was measured through cell count daily using a Neubaer haemacytometer. The total number of cells was calculated by using the following formula (Shahadat 2022):

$$\textit{Measured cell density} = \frac{\text{Average cells per small square . Dilution factor}}{\text{Value of a small square (mL)}}$$

Here,

Count (cell/ml) for 25 squares, 10 is the number of squares counted in 2 chambers, 4×10^{-6} is the volume of samples over the small squares area which is equivalent to 0.004 mm3 (0.2×0.2×0.1) expressed in cm^3 (ml).

Specific Growth Rate (SGR)

The obtained cell count data with different days were used to determine the Specific Growth Rate (SGR). SGR, µ was calculated using the following formula (Shahadat 2022):

$$\mu\left(\frac{\square}{\text{day}}\right) = \frac{\log X2 - \log X1}{T2 - T1}$$

Here,
μ = growth rate of per unit cell concentration,
X1 = cell concentration at the beginning of experiment (t1)
X2 = cell concentration at stationary day (t2)

Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) v. 20.0 for windows (SPSS, SAS Institute Inc. Cary, USA). The data were analyzed to determine the descriptive statistics such as Mean, Standard Error of Mean (SEM), Minimum & Maximum value and Ranges of variables. Independent sample t test and one-way ANOVA were performed at 5% level of significance. Microsoft excel (2013) was used to plot graphs.

Results and Discussion

All 3 studied species started cell division immediate after inoculation and reached to the peak on 9th day of culture. The stationary phase was observed for 9-14 days and then started to collapse. This type of growth pattern was observed in both indoor and outdoor condition. In outdoor culture condition, *Nannochloropsis* sp. showed highest growth performance ($3.03×10^6±1.40×10^6$cells/ml). whereas, in indoor condition *Tetraselmis* sp. exhibited highest cell growth ($6.67×10^6±2.20×10^6$ cells/ml) (Table 2).

Table 2. Average Growth performance (cells/ml) (Mean±SEM) of the three studied species under indoor and outdoor culture condition

Species Name	Indoor Growth (cells/ml)	Outdoor Growth (cells/ml)
Nannochloropsis sp.	$3.29×10^6±1.46×10^6$	$3.03×10^6±1.40×10^6$
Nannochlorum sp.	$5.03×10^6±0.25×10^6$	$2.30×10^6±1.66×10^6$
Tetraselmis sp.	$6.67×10^6±2.20×10^6$	$2.55×10^6±0.55×10^6$

The cell density was determined to evaluate the growth curve of the microalgae in different treatments. Microalgae growth stages are found as lag phase, log, or exponential phase, reducing growth rate phase, stationary phase, and death phase (Shahadat *et al.* 2022). The three species showed significantly different cell growth in indoor culture condition. In case of outdoor culture system, significant difference in growth performances were found between the two species *Nannochloropsis* and *Nannochlorum* (Fig. 1). A high resistance to contamination in long-term mass cultures of *Tetraselmis* sp. has already been reported (Pereira *et al.* 2018). This resistance pattern to contamination assists in uninterrupted cell growth of *Tetraselmis* sp. In the study of Cruz-y-Cruz *et al.* (2016), algae population densities were reached to the maximum

density of 3.1- 8×10^6 cells/mL in indoor culture using different types of fertilizer as culture nutrient. *Nannochloropsis oculata* cultures grew faster outdoors, producing more biomass within a shorter period of time (Banerjee *et al.* 2011). According to Fui *et al.* (2018), the highest density of *Nannochloropsis oculata* was attained from tilapia culture system 9.6×10^6 cells/ml harvested at day 7 while in pure culture system was 8.5×10^6 cells/ml harvested at day 4. This deviation may be caused temperature and various nutrient composition.
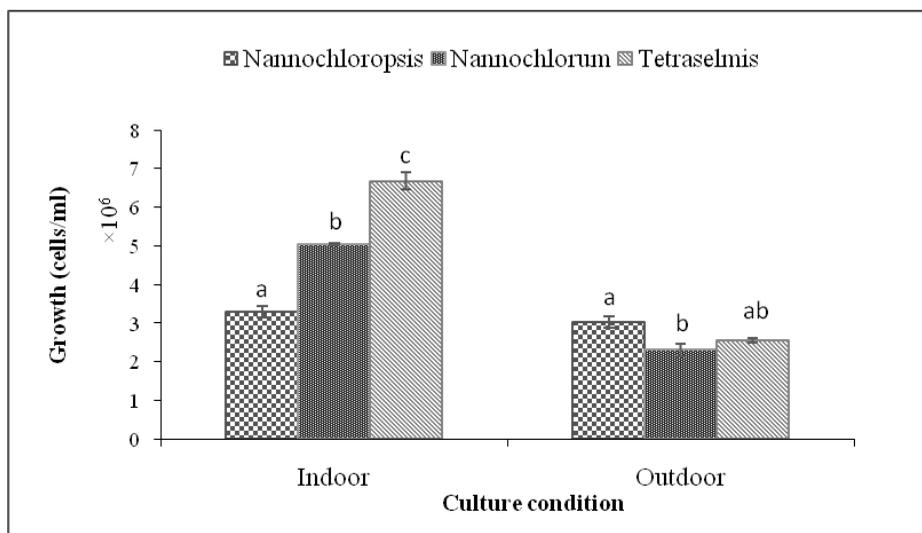
Fig. 1. Comparison of the average growth performance observed in both indoor and outdoor culture condition among the three Microalgae species.

In present study, all the studied microalgae species showed better performance in cell growth in case of indoor culture condition than outdoor. The indoor culture was conducted in a controlled environment so that the production was higher as expected. On the other hand, the outdoor mass culture was in open system and directly affected by the ambient environment. As a result, there was less control over the environmental parameters and the microalgae productivity was slightly lower than the indoor controlled environment. The highest cell densities of three microalgae were found in indoor culture condition (Table 2). In outdoor culture, the weather conditions are obviously factors with significant influences on growth performances. The temperature of the culture also greatly influences these results. (Gentile and Blanch 2000) established 25°C as a turning point for productivity in cultures of *Nannochloropsis gaditana*. Lopez *et al.* (2020) showed how productivities from cultivating microalgae in outdoor conditions are affected predominantly by seasonal changes. The productivity

values of outdoor microalgae culture are affected by conditions that cannot be controlled. According to the study of Moheimani and Borowitzka (2006), productivity values by area of up to 50 g m^{-2} day^{-1} can be achieved under controlled conditions in small indoor cultures, and up to 30 g m^{-2} day^{-1} in small outdoor raceways. These studies both previous and present reveal that there is a great effect of temperature and photoperiod in culture system of microalgae. The environmental parameters should be controlled considering the particular species. Open culture system has minimal control over culture environment (temperature, light etc.) and are more prone to contamination. Ambient air, rain and other common contaminants like algal predators (zooplankton), bacteria and competing strains can all reduce production efficiency or even spoil culture. So, mass culture in open system is environment dependent and ultimately the production varies with the fluctuations of the environmental parameters. For mass culture of algae with high productivity and commercialization of algae culture, the technology of photo bioreactors can be used in which a controlled environment can be maintained in outdoor.

The present finding concludes about the efficacy of indoor culture condition for microalgae culture over outdoor condition. From this experiment, it can be concluded that the feasibility of achieving better production of *Tetraselmis* sp. in indoor condition is comparatively higher (6.67×106±2.20 cells/ml) than the other two studied species of microalgae. Further research should be conducted using another experimental setup with different environmental parameters and culture condition to obtain the highest production feasibility of another two species. The new technologies today have led to the development of several types of closed photo bioreactors which will enable the commercialization of new algae and algal products. A good selection of microalgal species is also important to support the aquaculture industries. More research on other potential microalgal strains is recommended. Application of this findings might be developed the live feed production especially in the brackishwater hatcheries and extremely helpful for rearing of commercially important brackishwater finfishes and shellfishes. Combined effort to standardize a genetically modified microalgae aided with a controlled bioprocess system will lead to an improvement in the status of aquaculture.

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Length-Weight relationship, Gonadosomatic index and fecundity of Needle nose gar fish, *Xenentodon cancila* from Padma River of Bangladesh

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Abstract

An investigation of the length-weight relationship, gonadosomatic index (GSI), and fecundity of Needlenose gar fish, *Xenentodon cancila* was carried out for six months from March to August 2021 at the Freshwater Sub-station of Bangladesh Fisheries Research Institution in Jashore. As sample, 120 *X. cancila* adults were caught from the Padma River from in Kushtia and Pabna. The association between length and weight was isometric, and fecundity was positively correlated with increasing overall length, body weight, and gonad weight. The GSI varied from 3.45 to 9.46 and from 0.25 to 1.43 in female and male, respectively. In female, the highest GSI levels were found in the month of August and the lowest GSI levels were observed in the month of March.
Keywords: *Xenentodon cancila*, L-W relationship, GSI

Introduction

The Needle nose gar, *Xenentodon cancila* (Hamilton, 1822), is available in Bangladesh, India, Pakistan, Myanmar, Sri Lanka, Thailand (Talwar and Jhingran 1991), and Hawaii (Froese and Pauly 2012). However, it is a floodplain resident freshwater fish (IUCN 2015) but also survive in wide range of salinity thus it can be bring into being in fresh, marine and brackishwater (Hossain *et al*. 2013). Depending on the area it is called in various name *viz*. Kani/Kakila/Kaikka. The common name of gar is used for the reason that there are similarities between this fish and the true gars. Needlefishes are so-named since of their really extended jaws and bodies. This fish is very voracious and feeds mostly on animal products, fish, insects, and nematodes (Dasgupta 2008). It is used as food fish (Talwar and Jhingram 1991) having some medicinal value (Rahman 1989) which is a high priced fish in market. Though, *X. cancila* was formerly available copiously in various parts of Bangladesh (Mia *et al*. 2017) but its abundance has been declining day by day compared to other small indigenous species owing to environment obliteration and fishing stress (Islam *et al*. 2016). Therefore, the supervision of the fishery is essential for

the target fish, such as this garfish. The information of the diverse aspects of reproduction ecology such as maturity size, breeding season and fecundity are the fundamentals for equally culture and fisheries administration plan (Islam *et al*. 2016). Understanding of fecundity and spawning routine of a fish is also a significant aspect in stock size assessment, stock discrimination (Holden and Raitt 1974), non-availability of quality fish fry/fingerlings is one of the major constraints of fish culture in backyards area (*haor*) (Islam and Begum 2019). Therefore, conducting research on the reproductive performance of fish is crucial and essential for improving the management and sustainability of fisheries revenue streams (Marshall *et al.* 2003). In light of the aforementioned contexts, the purpose of this study is to investigate the propagation biology of garfish captured in the Padma River of Bangladesh, with the intention of contributing to the understanding and conservation of freshwater fish species.

Materials and Methods

The present study was conducted in the lower parts of the Ganges River, North-Western (NW) Bangladesh also known as the Padma River. Three sampling spots were fixed for the fish collection from Pabna and Kushtia district. The sampling spots were Ambaria and Shelaidaha in Kushtia district and Char Bhabanipur in Pabna district. Name of the sampling stations with GPS point are shown in Fig. 1. Experimental fish samples were collected monthly from March to August 2021.
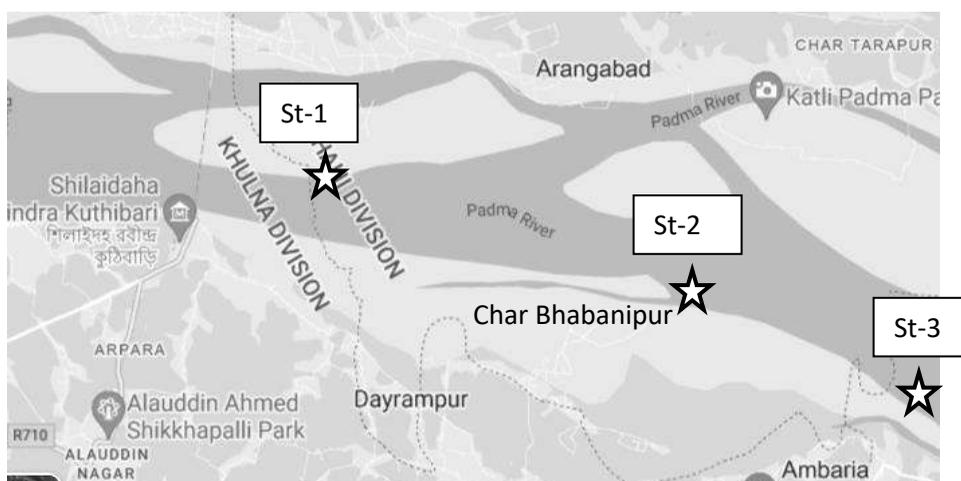

Fig. 1. Location of sampling stations (indicated by yellow stars).

Length-weight relationship
The length-weight relationship was estimated according to power equation (Froese 2006) as follows: W = a × TLb
where, W= total body weight (g), TL= total length (cm), and 'a' and 'b' are constants.
To determine the coefficient of determination (R^2) values as well as to determine the mathematical link between fecundity and total length, fecundity and body weight, fecundity and gonad weight, a specific statistical formula was applied (Achakzai *et al*. 2013):

Y= a + bX
where, Y = Fecundity, X = total length (cm) or body weight (g) or gonad weight (g), 'a' & 'b' are regression constants.

Gonado- Somatic Index (GSI)
The Gonado-somatic Index (GSI) was premeditated to determine the spawning time of year in together male and female. GSI was calculated on monthly basis by following standard method (Le Cren 1951). For this, the ovary was calculated very vigilantly with the assist of a handy electronic balance (Model FX- 300). The gonads were removed by abdominal dissection and weight was taken.

$$\text{The Gonado somatic Index (GSI\%)} = \frac{\text{Weight of gonad}}{\text{Weight of fish}} * 100$$

Fecundity estimation
The fecundity was computed with the Von Vayer formula. The fish were immediately sent to the lab for a detailed review of fecundity. The overall length of each fish was checked with an analogue scale to the nearest millimeter after meticulous washing in tap water, and the body weight in grams was determined using an electronic balance (Model FX-300). Before we could weigh the fish, extra water from them had to be blotted out with paper. The ovaries' outer connective tissues were cut out, and the gonads were taken out with scissors. The ovaries were weighed using an electronic scale (Model FX-300), and any additional water was absorbed using a paper towel. After accurately extracting 10 mg from each gonad, the ovary was fixed in buffered 10% formalin for 12 hours, kept in 70% ethanol, and gently shook to ensure a homogeneous mixture. The entire quantity of eggs was calculated by dividing the weight of the ovary by the average number of eggs counted overall.

$$\text{Fecundity} \frac{\text{Number of eggs in the fraction} \times \text{Total weight of ovary}}{\text{weight of the fraction}}$$

Statistical analysis

Microsoft Excel was used to store all of the captured data. Microsoft Excel 2013 and Statistix 10 served as tools with a 5% level of significance to identify linear and non-linear relationships and coefficient of determination (R^2).

Results and Discussion

Length-weight relationship (LWR)

A total of 120 specimens of *Xenendon cancila* were analyzed for this study. The female fishes were found larger than males in both total length and weight. Sexual dimorphism was obvious as males possess black edge at dorsal and anal fins whereas females lack this character. Logarithmic linear regression relationship of pooled data between total length and body weight of *X. cancila* over the study period was estimated as Log BW = 0.0707(Log TL) + 0.9275 (Fig. 2). The intercept "Ln a" was 0.09275 and slope "b" was 0.0707 for this species which indicating the pattern of negative allometric growth of this species as b<3. It means that they are favor to increase in length than in mass in although variation in the Length-weight relationship may depends on the population, season and environmental conditions (Froese 1998). Khoso *et al.* (2018) studied positive allometric growth for combine sex on length-weight relationship of *X. cancila* and recorded higher than isometric growth of the fish (b>3), which showing positive allometric growth. The value of b can also be affected by ecological factors. Which comes healthy environment condition just as temperature, pH, light, sex, fishing and breeding (Ricker 1973). The positive allometric growth in the species indicates that there is sufficient food in aquatic medium. The length and weight were determined separately for combined, male and female population of *X. cancila*. The length and weight relationship also depends on seasonal variations and the fish is unable to maintain the same shape throughout of the year, so there will be changes in slope values. The findings of various researchers suggested that growth is affected by seasonal changes, spawning period and other environmental factors (Sinha 1973). The growth may also be affected by gonads and age of the fish (Tesch 1968). The present concluded that the growth of *X. cancila* in Padma river's water is not ideal. There is scanty of food or have any other disturbance for optimal growth of the niddlenose gar fish. Chakraborty *et al.* (2019) estimated length weight relationship on *Mystus vittatus* in two different aquatic habitats and found a negative allometric growth. With the regression coefficient (b) of 2.71, a negative allometric growth has been reported for *M. cavasius* from natural catch (Latif *et al.* 2018). The coefficient of determination R^2 was found 0.94 indicating a good linear regression between length and weight of the species.
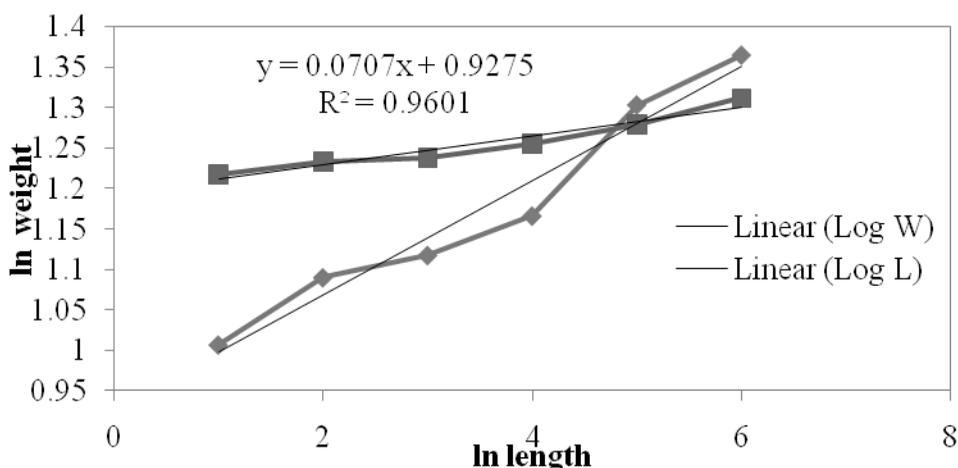
Fig. 2. Relationship between total length and body weight of *Xenendon cancila*.

Gonado somatic index (GSI)

Every month throughout the research period, the gonado-somatic index (GSI) of the ovary and testis of *X. cancila* was documented. Fig. 3 presents the findings. The month of August had the male and female gonads' greatest GSI levels throughout the study period, while the month of March saw the least GSI levels. This variance may be related to the fish's ability to reproduce at various times of the year and the maturation of their eggs. Mean GSI levels were greater in June and July even though the peak GSI value was at its highest in August. The mean Gonado-somatic index of the ovaries and testicles was shown to gradually rise from March to August, reaching its highest in that month. The reported result demonstrates the fish's high metabolic activity from May to August, when they are spawning. It was discovered throughout the research session that the months of July and August are when fish spawn the most. Over the pre-spawning period, which lasts from March to April, the gonado-somatic index is visible and is shown in an upward sequence. It demonstrates that the oogenesis phase has proceeded and that the ovaries are now active. The fact that GSI rises during the mating season is well established. The percentage of developed eggs with yolks was higher in the times of June, July, and August, as shown by the freshwater garfish's growing GSI value. Sarker *et al*. (2002) found similar sorts of findings in *Mystus gulio*, while Das *et al*. (2023) reported them in *Neotropius atherinoides*. In the current investigation, it was shown that the testicular GSI values in *X. cancila* follow a predictable cyclical pattern. March had the lowest GSI value recorded. As the testes begin to mature in May, the GSI value rises quickly until it reaches its peak in August.

Fecundity

Fecundity refers to a fish's ovary's capacity to produce eggs. The word "fecundity" refers to a fish's ability to lay eggs or the total quantity of mature eggs that a fish produces during one spawning season. For the purpose of assessing a fish's commercial potential, life history, practical culture, and real supervision of the fishery, comprehension of fecundity is crucial. From March to August 2021, fish fecundity in relation to length and body weight was investigated. Table 1 presents the findings.
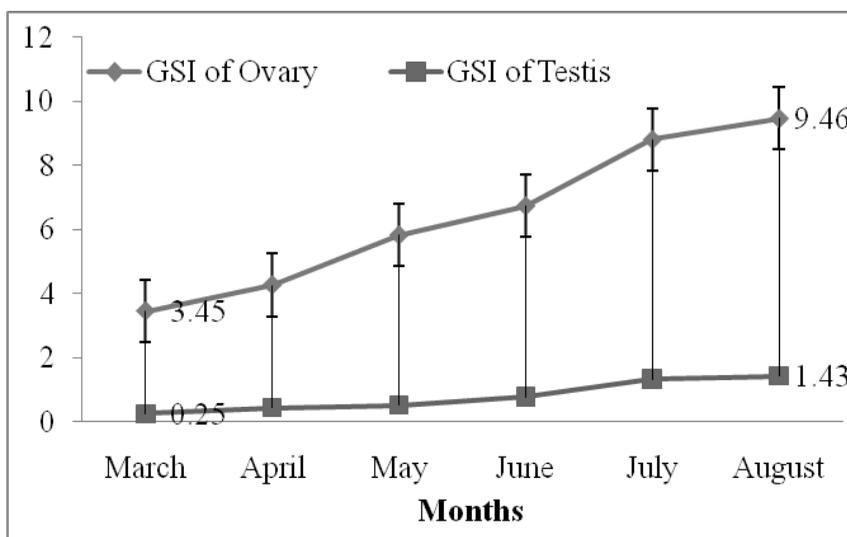

Fig. 3. GSI of Ovary and Testis of *X. cancila* during study period

In the current investigation, it was discovered that when overall length, body weight, and ovary weight increased, so did the quantity of eggs. Fecundity and total length, body weight, and ovary weight are all extremely important connections. The findings of earlier researchers (Hussain *et al.* 2003) are consistent with our results. It was clear from the regression equation that total length and fecundity had a positive association with each other for the fish species *X. cancila*. The results of Hussain *et al.* (2003) for *Osteobrama cotio,* Mohan (2005) for snow trout, and Bahuguna and Khatri (2009) for *Noemacheilus montanus* are in agreement with this conclusion. Body weight and fecundity were shown to be positively and linearly correlated by the regression equation. This discovery is consistent with those made by Rishi and Kaul (1982) and Misra (1994). It was discovered throughout the investigation that varying quantities of eggs were present in the ovaries of fishes of the same size. This could be caused by changes in the individual's diet and environmental factors. Numerous studies have

noted the frequent occurrence of fecundity variation in fish (Bhuiyan *et al*. 2006). To date, it has been reported that a variety of factors, including different fish stocks, nutritional status, racial characteristics, sampling time and stage, and changes in environmental parameters (Bhuiyan *et al*. 2006), might impact fecundity both within and between fish populations. Consequently, the study's variable fecundities were not an outlier. The current research shows that the *X. cancila* is a poor fecund fish since only 135 to 205 eggs are laid per fish. Due to its dependence on a variety of variables, such as fish stock, nutritional condition, and racial features (Das 1977), such as size, age, sex, environmental circumstances, and the abundance of space and food (Hunter 1992), the variation in fecundity is widespread in fish (Reddy and Rao 1991).

Table 1. Showing total length (cm), body weight (gm), fecundity of mature *X. cancila* fish during study period (n=20)

Months	No. of fish examined	Body length (cm)	Body weight (g)	Fecundity
March		16.51±0.22	11.87±0.18	135±5
April		17.33±0.23	12.91±0.15	145±8
May	20	18.2±0.25	16.32±0.18	152±9
June		19.03±0.29	15.0±0.13	171±10
July		18.0±0.31	14.66±0.19	195±10
August		20.5±0.43	23.15±0.29	205±13

Fecundity's relationship to other factors
Fecundity of *X. cancila* ranges from 135 to 205 in the current research. Fish length and weight are shown to be strongly correlated with a fish's fertility. According to the research, fecundity considerably rises as overall length rises. It rises linearly. Fig. 4 shows the formula for the regression, which is Y=18.703x-170.77 and has a significant R^2 value of 0.9446 when a linear curve has been matched to the data being analyzed.
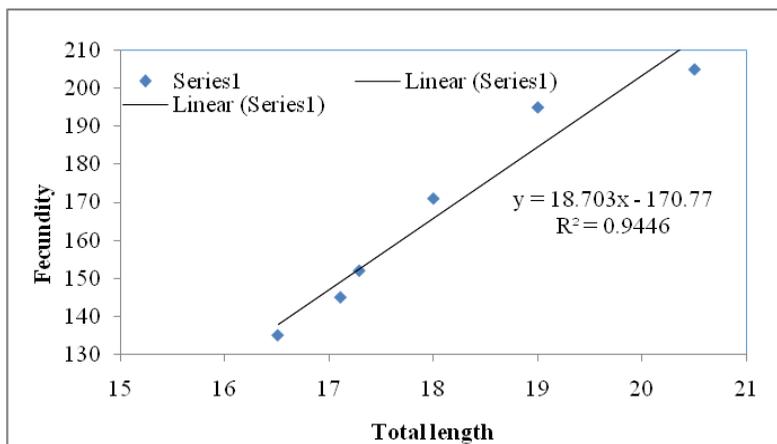
Fig. 4. Relation between fecundity and total length.

The current research also discovers that fertility increases as body weight increases. Fig. 5 shows the regression line, which is y=5.557x+80.57 and has a significant R^2 value of 0.9679, when a linear line is shown against the data. Thus, it can be shown that a fish's fertility grows linearly as ovary weight increases. According to Gupta (1967), there are numerous fish species where fecundity and body weight are positively correlated. As gonad weight grew, fecundity rose. This outcome is also supported by Sultana (2010). The link between fecundity and gonad weight was extremely favorable since it showed a rise in fecundity as gonad weight grew up to gonad maturation.
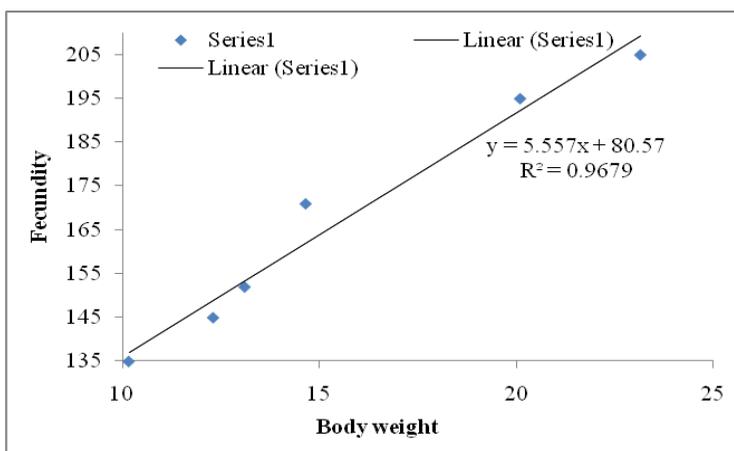

Fig. 5. Relation between fecundity and body weight.

The current research also identifies an association between ovary weight and fecundity. It rises linearly. In Fig. 6, the regression line is shown to be Y=0.0256x-3.18, with a significant R^2 value of 0.9743. Thus, it can be shown that a fish's fertility grows linearly as ovary weight rises.
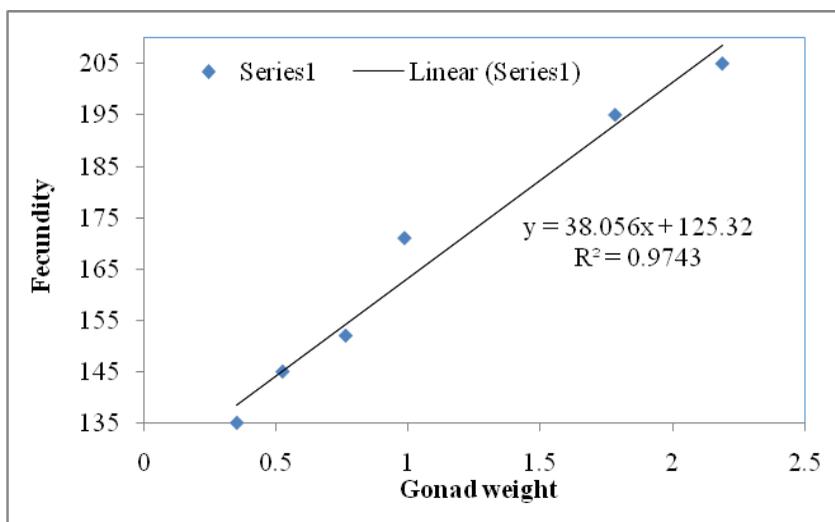

$$y = 38.056x + 125.32$$
$$R^2 = 0.9743$$

Fig. 6. Relation between fecundity and gonad weight.

Based on the findings of this inquiry, it can be concluded that *X. cancila* reproduces from May to August. Observations indicate that the number of spawning fish is greatest in July and August. Compared to other species of fish, the fertility rate of *X. cancila* is quite low.

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Role of dietary phospholipid on Mud crab (*Scylla olivacea*) larvae reared in hatchery conditions

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Abstract

An experiment was conducted to explore the effects of dietary phospholipids (PLs) on LSI, growth and survival rate of mud crab (*S. olivacea*) larvae rearing, three different levels of PLs *viz.*, T_2= (0.25 µg.kg^{-1} feed or L^{-1} water); T_3= (0.5 µg.kg^{-1} feed or L^{-1} water); and T_4= (0.75 µg.kg^{-1} feed or L^{-1} water) was tested. A set of controls (T_1= no PLs) was assigned for comparison. Each of the treatments had three replications, stocking density was 50 larvae L^{-1} and experimental duration was 27 days to achieve crab instars. Before 12 hours, the corresponding doses of PLs were added to the live feed (rotifer and artemia). Feeding was done 6 times a day with rotifer for Z1 to Z2, with artemia for Z3 to Z5 stages, and chopped trash fish at the later stage of megalopa onwards. All three treatments exhibited better larval stage index (LSI) than the control and LSI in T_3 was superior to the other two levels (T_2 and T_4). The highest survival of 6.4% was observed in T_3 and it was statistically insignificant ($p>0.05$) with T_2 (5.2%) and T_4 (4.8%) but significantly differed ($p<0.05$) with T_1 (3.2%). Larvae size was similar up to the Z2 stage and then started to vary among treatments. The highest carapace length (3.32 mm) and carapace width (1.62 mm) in the megalopa stage and the highest carapace length (2.89 mm) and carapace width (3.40 mm) in the crab instar stage was observed in T_3 followed by T_2 and T_4. However, the lowest carapace length and carapace width were in T_1 (control). Results of the present study suggested a level of 0.5 µg.kg^{-1} feed or L^{-1} water of dietary phospholipids for successful mud crab larvae rearing and conducting multi-dimensional research to enhance the survival of mud crab larvae.

Keywords: *Scylla olivacea*, Mud crab, Rotifer, Phospholipid

Introduction

The orange mud crab, scientifically known as *Scylla olivacea* (Herbst 1796), is a widely distributed species that is abundant in the marshes around the Sundarbans, the biggest mangrove forest in the world (Khatun *et al*. 2009). *S. olivacea* is well recognized as a promising species for aquaculture due to its excellent nutritional value and thrilled market demand (Muhd-Farouk *et al*. 2019). According to Asaduzzaman *et al*. (2021), in Bangladesh, *S. olivacea* is part of a larger group of mud crabs compared to *S. serrata*

(Sakib *et al*. 2022). In recent times, mud crabs have become widely utilized for both commercial and therapeutic purposes in various nations across the globe. This is mostly owing to their exceptional nutritional value and the presence of antioxidant capabilities, as highlighted by Yusof *et al*. (2019). Furthermore, *S. olivacea* has gained significance as a valuable aquatic resource for export, second only to tiger shrimp (*Penaeus monodon*).

Several nutritional studies have shown that phospholipids (PLs) are an essential nutrient for marine crustaceans and finfish. They have positive effects on growth, survival, nutrient utilization, skeletal/organ development, molting performance, immunity and ability to withstand stress in certain fish and crustaceans (Pascual 1986, Gong *et al*. 2000, Roy *et al*. 2006, Uyan *et al*. 2007, Wu *et al*., 2007, 2010, 2011). Some researchers have proposed that the positive benefits of PLs cannot be duplicated by supplementing with choline, inositol, phosphorous, and fatty acids (Coutteau *et al*. 1997, Tocher *et al*. 2008). In addition, the production of PLs by de novo synthesis is insufficient to fulfill the metabolic needs of crustaceans and finfish (Kanazawa 1985, Tocher *et al*. 2008). As a result, PL is extensively utilized in the diets of crustaceans and fish. Nevertheless, the expensive nature of purified PL has resulted in most feeding studies mentioned in the literature being conducted with PL of variable or low purity (Pascual 1986, Teshima *et al*. 1986, Kanazawa *et al*. 1979). Tocher *et al*. (2008) indicated that various phospholipid classes may have distinct functions and consequences in different animals. Moreover, multiple studies have also demonstrated that various sources of PLs have distinct impacts on fish and crustaceans (Coutteau *et al*. 2000, Hamza *et al*. 2012, Azarm *et al*. 2013). Therefore, it is challenging to make definitive determinations regarding the sources, quality and quantity prerequisites of dietary PLs for crustaceans and finfish. Extensive studies have been undertaken on the dietary phospholipid requirements in crustaceans such as *Litopenaeus vannamei* (Gong *et al*. 2000, Roy *et al*. 2006), *Penaeus merguiensis* (Thongrod and Boonyaratpalin 1998), and *Eriocheir sinensis* (Wu *et al*., 2010, 2011). In a study, Mokoginta and Suprayudi (1996) found that adding PLs to the diet resulted in increased survival and growth of *Penaeus monodon* larvae and post-larvae. The early juvenile stage is a crucial transitional time in the growth of crabs. Research conducted previously has shown that crustaceans exhibit a greater need for PLs during their early juvenile stages compared to their later stages (Wang *et al*. 2018). Hence, this research aimed to ascertain the most favorable amount of dietary PLs supplementation for young *S. olivacea* by assessing the larval stage index (LSI), survival rate, and growth performance.

Methodology

Larvae management

The study utilized healthy broodstock of *S. olivacea* that were sourced from nearby rivers of Paikgacha, Khulna, Bangladesh. The broodstock was then placed in a 5-kilo fiberglass tank that was equipped with a flow-through water system. A female bearing berry was moved to a 1,000-litre tank made of polycarbonate for about 10 days for embryogenesis. The tank was aerated and the salinity was kept at 34 ppt, while the temperature was maintained at 26°C. One hour after the eggs hatched, the larvae that were swimming actively were taken out and utilized in the experiment.

Larvae were stocked at a rate of 50 larvae L^{-1} plastic buckets. A salinity of 33–34 ppt was maintained in the water throughout the zoeal stages' raising period. Following the larvae's transformation into the Megalopa stage, the salinity was gradually dropped and regulated at 24 ppt. A regulated heater was used to keep the water temperature at 30°C. A Pasteur pipette was inserted into the center of each bucket to gently aerate the contents. Using a 5 ml pipette, the larvae were moved into fresh buckets every morning. Larvae were counted and developmental stages were noted during the transfer. The larvae were sorted into distinct buckets based on the stage at which they were.

Enrichment of rotifer and Artemia with phospholipid

The experiment was conducted with three distinct treatments, each varying in the amount of PLs administered. Specifically, T_2 consisted of 0.25 $\mu g.kg^{-1}$ of PLs in the feed or liter of water, T_3 consisted of 0.5 $\mu g.kg^{-1}$, and T_4 consisted of 0.75 $\mu g.kg^{-1}$. A control treatment set (T_1) without PLs was assigned for comparison. Each treatment was replicated thrice, and the stocking density was 50 larvae L^{-1}. The live feed, consisting of rotifer and artemia nauplii, was enhanced with specific doses of PLs for each treatment, at least 6 hours before feeding. The duration of the experiment was 27 days to reach the crab instars.

The larvae were provided with a daily diet of rotifers (40 individuals ml^{-1}) from the first zoea (Z1) stage until the second zoea (Z2) stage. The density of Artemia was 1.5 nauplii ml^{-1} when Z3 larvae were fed and rose to 4 nauplii ml^{-1} at the megalopa stage.

Evaluating parameters

Survival rate, carapace width in the megalopa and first crab stage, and larval stage index (LSI) to the megalopa stage were measured for the larvae in this experiment. The survival rate was determined by calculating the percentage of larvae that successfully

underwent molting from the first zoeal stage. The carapace width was estimated by measuring the outer lateral spines and distinguishing the variations between zoeal stages by comparing the distance between the eyes using a microscope equipped with a micrometer (50x) (Suprayudi *et al*. 2012). Larval stage index (LSI) was calculated by the following equation (Syafaat *et al*. 2019):

LSI = ((A1xA2) + (B1xB2))/ C,
Where, A1 = amount of previous stage larvae; A2 = previous stage; B1 = amount of highest stage larvae; B2 = highest stage; C = total amount of sample.
SR (%) = (N_t/N_0) *100
Where, SR = Survival rate of crab; N_0= Number of crabs at the beginning of the study; N_t= Number of crabs at the end of the study

Statistical analysis
The data obtained were generally organized in MS Excel and examined to produce the research findings. The comparison of the various experimental findings was assessed using one-way ANOVA and DMRT (Duncan 1955) as required. The statistical analysis of the biometric parameters among the set of treatments was conducted using two computer programs: Microsoft Office Professional Plus 16 and SPSS ver. 20.

Results

Water quality parameters
The water quality data for various treatments during mud crab larvae rearing is displayed in Table 1. The water temperature of the tanks used for growing larvae varied from 28.85°C to 31.54°C. The levels of dissolved oxygen, pH, NO_2, and total NH_4 in all treatments varied between 5.14 and 5.86 mg l^{-1}, 7.49 and 8.4, 0.04 and 0.09 mg l^{-1}, and 0.37 and 0.55 mg l^{-1}, respectively. The mean values of water quality variables did not exhibit any significant ($p>0.05$) variation among the treatments, and all the parameters remained within the suitable range for the cultivation of crustacean larvae.

Larval stage index (LSI)
The larval stage index (LSI) of the larvae as shown in Table 2, demonstrating synchronized growth for up to 6 days of development. The variation in LSI among the treatments began on the 9th day of rearing. The treatments treated with PLs had a higher LSI compared to the control and T_3 had the highest LSI among the three levels.

Table 1. Water quality ranges for crab larvae rearing under different treatments

Parameters	Time	Treatments				Range
		T_1	T_2	T_3	T_4	
Temperature (°C)	8:00	29.45±0.5	29.85±0.6	29.85±0.4	29.85±0.4	28.85-31.54
	14:00	31.54±0.5	31.04±0.5	29.82±0.6	29.9±0.6	
Dissolved oxygen (mg l^{-1})	8:00	5.16±0.2	5.20±0.1	5.21±0.2	5.22±0.1	5.14-5.86
	14:00	5.41±0.3	5.47±0.4	5.46±0.4	5.42±0.4	
pH	8:00	7.99±0.5	8.07±0.3	8.1±0.2	8.10±0.3	7.49-8.4
NO_2 (mg l^{-1})	8:00	0.05±0.01	0.06±0.02	0.07±0.02	0.04±0.03	0.04-0.09
Total NH_4 (mg l^{-1})	8:00	0.4±0.03	0.51±0.04	0.4±0.02	0.41±0.04	0.37-0.55

Data are expressed as mean ± standard deviation (n=3)

Table 2. The larval stage index (LSI) of larvae measured on different days under various treatments

Treatment	Larval Stage Index (LSI)					
	Day 3	Day 6	Day 9	Day 12	Day 15	Day 21
T1	1.7±0.1	2.5±0.1	3.6±0.1[dcb]	4.6±0.1[cb]	5.5±0.1[cd]	6.5±0.1[cb]
T2	1.9±0.1	2.6±0.1	3.8±0.1[cba]	5.0±0.1[a]	5.8±0.1[ba]	6.7±0.1[ba]
T3	1.9±0.1	2.7±0.1	4.0±0.1[a]	5.0±0.1[a]	5.9±0.1[a]	6.8±0.1[a]
T4	1.7±0.1	2.5±0.1	3.8±0.1[ba]	4.8±0.1[ba]	5.6±0.1[cb]	6.7±0.1[ba]

Data are expressed as mean ± standard deviation (n=3). Different superscripts in the same column indicated significant differences ($p < 0.05$) between treatments; shared superscripts indicated insignificant ($p > 0.05$) and a > b > c > d.

Growth of mud crab larvae

The dimensions of the larvae at various developmental stages are provided in Table 3. The size of the larvae was consistent at the time of stocking and remained consistent until the Z2 stage, after which it began to differ among the different treatments. The megalopa stage exhibited the greatest carapace length (3.32 mm) and carapace breadth (1.62 mm) in T_3, followed by T_2 and T_4. Similarly, the greatest carapace length (2.89 mm) and carapace breadth (3.40 mm) in crab instars were also seen in T_3, with T_2 and T_3 following closely behind. Nevertheless, the smallest measurements for carapace length and carapace breadth were seen in T_1 (control).

Table 3. Variations in size of mud crab larvae at different larvae stages under different treatments

Larvae Stage[1]	Larvae size (mm)			
	T_1	T_2	T_3	T_4
Z1	1.50±0.041	1.50±0.041	1.50±0.041	1.50±0.041
Z2	2.17±0.02	2.19±0.05	2.19±0.08	2.19±0.02
Z3	2.68±0.04[cd]	2.82±0.04[ba]	2.87±0.04[a]	2.72±0.05[c]
Z4	2.82±0.05[dcb]	2.90±0.04[ba]	2.92±0.03[a]	2.87±0.07[cba]
Z5	3.64±0.04[cd]	3.71±0.01[ba]	3.74±0.02[a]	3.67±0.03[cb]
MCL	3.30±0.04[dcba]	3.31±0.01[ba]	3.32±0.03[a]	3.30±0.04[cba]
MCW	1.55±0.02[dcba]	1.58±0.03[ba]	1.62±0.02[a]	1.56±0.03[cb]
CCL	2.75±0.04[dc]	2.84±0.04[ba]	2.89±0.02[a]	2.76±0.04[cb]
CCW	3.18±0.06[dc]	3.32±0.05[ba]	3.40±0.03[a]	3.21±0.06[cb]

Data are expressed as mean ± standard deviation (n=3). Different superscripts in the same row indicated significant differences ($p < 0.05$) between treatments; shared superscript indicated insignificant ($p > 0.05$) and a > b > c > d; and Z1 = Zoea 1, Z2 = Zoea 2, Z3 = Zoea 3, Z4 = Zoea 4, Z5 = Zoea 5, MCL= Megalopa carapace length, MCW = Megalopa carapace width or breadth, CCL = Crab-instar carapace length CCW= Crab-instar carapace width or breadth.

Survival rate of mud crab larvae

The survival of larvae at various stages is presented in Table 4. The bulk of the larvae perished during the Z1 and Z2 stages. The survival rates of megalopa and crab-instar stages were higher in the treatments with phospholipid therapy compared to the control group. The highest survival rate of 6.4% was seen in T_3, and it was not statistically significant ($p > 0.05$) when compared to T_2 (5.2%) and T_4 (4.8%). However, the survival rate in T_3 exhibited a statistically significant difference when compared to T_1 (3.2%).

Table 4. Variations in size of mud crab larvae at different larvae stages under different treatments

Treatments	Survival (%)[1]						
	Z1	Z2	Z3	Z4	Z5	M	C
T_1	76±4.0[a]	64.2±2.8[dcb]	39.5±4.7[cba]	22.5±5[dcba]	11.7±2.9[d]	7.9±2.5[cd]	3.2±1.1[dc]
T_2	78±4.0[a]	66.8±5.4[ba]	38.5±3.6[dcba]	26.7±5.8[ba]	20.0±1.4[b]	12.8±1.5[ba]	5.2±0.6[ba]
T_3	80±4.0[a]	70.6±1.2[a]	44.5±5.7[a]	31.7±6.3[a]	29.3±5.7[a]	15.5±5.7[a]	6.4±1.4[a]
T_4	78±4.0[a]	65.0±4.8[cba]	42.7±2.1[ba]	23.8±4.8[cba]	19.0±2.0[bc]	11.3±2.7[cba]	4.8±1.1[cba]

Data are expressed as mean ± standard deviation (n=3). Different superscript in same column indicated significant difference ($p < 0.05$) between treatments; shared superscript indicated insignificant ($p > 0.05$) and a > b > c > d; and Z1 = Zoea 1, Z2 = Zoea 2, Z3 = Zoea 3, Z4 = Zoea 4, Z5 = Zoea 5, M= Megalopa, C= Crab-instar

Discussion

Similar to other crustacean species, the growth of mud crabs is facilitated by molting. This process can be influenced by various circumstances, such as temperature, stress, predator threats, limited hiding places, inadequate nourishment, and hydrology. Any disruption in the molting process can impede the growth of mud crab larvae (Kulmiye and Mavuti 2005). The water quality parameters are crucial for all aquaculture techniques, particularly for the maintenance of healthy larvae (Gunarto and Sulaeman 2017). Optimal water quality parameters contribute to increased growth rate, survival, and production in the raising of mud crab larvae (Ong *et al*. 2019). The water quality variables in all treatments were nearly identical and felt within the range allowed for growing *S. olivacea* larvae. The water quality metrics throughout seed generation and larval rearing of mud crab in this study exhibited a high level of consistency, as documented by previous researchers (Chaoshu and Shaojing 1992, Dat 1999, Gunarto and Sulaeman 2017). Optimal water parameters create the ideal conditions for the generation of seeds and the rearing of larvae of *Scylla sp.*, resulting in high survival rates and rapid metamorphosis (Quinitio 2009).

PLs affected the larval stage index (LSI) of mud crab *S. olivacea*. Crab-instar was achieved at 21 days where T_3 had the highest value of LSI. LSI of T_3 though did not differ significantly from other treatments of phospholipid (T_1 and T_2) but did vary significantly from T_1 (control). LSI of crab changes with the molting performance. When the molting synchrony occurs results in rapid changes to next level of LSI. As the values of LSI especially T_3 was significantly ($p<0.05$) different from that of control which means molting synchroneity occurred due to the use of phospholipid (Suprayudi *et al*. 2012a). Other studies also found significant positive result using PLs, HUFA, PUFA, EPA, DHA and cholesterol on the molting synchrony of other crustaceans which are indicates LSI (Syafaat *et al*. 2019, Wang *et al*. 2016, Li *et al*. 2014, Suprayudi *et al*. 2012b).

PLs in the current study affected the growth and survival of mud crab larvae. Various researches has indicated that an appropriate level of dietary PLs can enhance the growth and survival of certain aquatic crustacean species (Xu *et al*. 1994, Suprayudi *et al*. 2004, Tocher *et al*. 2008, Li *et al*. 2013, Li *et al*. 2014, 2016, Wang *et al*. 2018). The crabs in the T_3 group exhibited considerably greater growth and survival in all phases of development compared to the other treatments ($p<0.05$). The findings were in line with prior research for early juvenile *Portunus trituberculatus* (Wang *et al*. 2018), and for post-larvae of *Penaeus monodon* (Briggs *et al*., 1994). Different species of crustaceans require different amounts of PLs. Studies have shown that certain crustaceans, such as juvenile *P. trituberculatus* and juvenile *P. monodon*, have reduced dietary PL requirements. For

example, a study found that juvenile *P. trituberculatus* required 1% phospholipid (Li *et al*. 2014), whereas another study found that juvenile *P. monodon* required 1.25% PL (Chen 1993). Thus, young *S. olivacea* may likewise experience a comparable condition. Crustaceans exhibit a greater need for PLs during their early life stages, as demonstrated by Coutteau *et al*. (1997) and Thongrod & Boonyaratpalin (1998). Many aquatic species have a restricted ability to produce PLs from scratch during their larval and early juvenile stages (Kanazawa 1993, Coutteau *et al*. 1997, Thongrod and Boonyaratpalin 1998). In addition, the digestive system of aquatic animals is not fully developed during the early stages of their existence. PLs have a greater utilization rate compared to neutral lipids, both for providing essential fatty acids (EFA) and energy. This has been seen in studies conducted by Koven *et al*. (1993) and Olsen *et al*. (1991). Though, Li *et al*. (2016) did not find any significant effect of phospholipid on the growth and survival of juvenile swimming crab *P. trituberculatus*.

The addition of PL through live feed certainly enhanced the LSI, size and survival of mud crab larvae rearing in hatchery conditions. However, a dose of 0.5 µg.kg^{-1} or l^{-1} dietary PLs supplementation had a beneficial effect on the larval stage index (LSI), molt performance, growth and survival on larval rearing of *S. olivacea*. This study would be of greater help to the mud crab hatchery operators in Bangladesh.

Acknowledgment

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Growth performance and nutritional efficiency of selected live feed cultured under different feeding regime for brackishwater hatchery

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Abstract

Microalgae plays vital role in larvae rearing of coastal fish, shrimp, prawn and crab in hatcheries. In this study 16 species of brackishwater microalgae has been identified under microscopic study. Off which two (*Nannochloropsis oculata* and *Spirulina platensis*) has been isolated and cultivated in hatchery conditions under different culture media. *N. oculata* growth was observed in 12-days culture with F2 media, cell density gradually upgraded quantitatively and reached to the highest pick ($59.70\pm6.50\times10^6$ cells/ml) in 7th day during 2nd trial then collapsed in next day as the addition of media was seized. *S. platensis* was cultivated with three different media and culture cycle was also 12 days. In the 6th day, highest cell density in Kosaric media (KM), Zarok media (ZM) and Bangla media (BM) were $32.37\pm1.23\times0^6$ cells/ml, $34.68\pm2.94\times10^6$ cells/ml, $21.30\pm0.99\times10^6$ cells/ml, respectively. Average highest cell density of *N. oculata* was $46.50\pm11.97\times10^6$ cells/ml and average highest cell density of *S. platensis* was $28.59\pm3.93\times10^6$ cells/ml, $32.59\pm3.04\times10^6$ cells/ml and $19.92\pm1.81\times10^6$ cells/ml under KM, ZM and BM, respectively for three successive trials. The protein content in *S. platensis* showed better levels ($57.44\pm0.32\%$) than *N. oculata* ($53.75\pm0.32\%$). In the case of rotifer, maximum population growth (114 ind/ml) was observed at the end of 8th day culture period when fed with live *N. oculata*, which was comparatively higher than the Baker's yeast (84 ind./ml) and *Spirulina* powder (78 ind./ml). Meanwhile, the nutrient content of rotifer, which was enriched with fish oil, showed superiority in terms of protein (51.2%) and lipid (25.5%) than that of the *Nannochloropsis* or *Spirulina* fed groups. Result of this study suggested to grow the rotifer with either Baker's yeast, *Spirulina* or *Nannochloropsis* and subsequently fortified with fish oil before feeding to support nutritional requirement of fish, shrimp and crab larvae in hatcheries.
Keywords: Microalgae, Live feed, Rotifer, Brackishwater hatchery

Introduction

Micro-algae are an important and crucial factor for the commercial culture of various species of marine animals as a food source for all growth stages of bivalve mollusks, larval stages of some crustacean species, and very early growth stages of some fish species. Again, microalgae offer rich sources of protein and lipids. The vitamins and pigments found in microalgae are of better quality and are healthier. The nutritional

value of live food organisms plays a significant role in the growth and survival of cultured species. Microalgae are fed to or are prey of larval and post larval stages of fish, abalones, sandfish, shrimp, and other crustaceans. They can be given directly as pure algal cells or in processed from which they are incorporated in formulated diets for fish rearing. High lipid microalgae are used to enrich rotifers to improve growth, survival and nutrition.

The larvae of several major species such as shrimp, mullet, Scats and Grunters are raised in captivity where rotifer is the principal initial food. The rotifer's small size and slow swimming velocity make it a suitable prey for fish larvae. It was suggested that coastal fish larvae that have only a partially developed digestive tract after hatching depend strongly on exogenous enzymes, provided by the live food they consume, for digestion of their prey. This means that rotifers or *Artemia* are partly digested by their own enzymes that are released when they reach in the gut of the larvae (Lauf and Hofer 1984). Furthermore, rotifers can be used for transferring probiotic bacteria to fish larvae (Gatesoupe 1999, Markridis *et al*. 2000). For the rotifers it is also important to consider successful micro-algae collection and proper culture. Because of that, it also progresses rotifers survivability which is direct feed for several fish larvae and in this development stage larvae also feed micro-algae directly which is reported to be most of the hatchery operation. Information regarding the culture and nutritive value of live feed in Bangladesh context is scares. However, this paper addresses to establish the culture protocol and assessment the nutritional quality of some important live feeds isolated from local sources.

Materials and Methods

Study location and duration
Consecutive experiments were carried out in the live feed laboratory and hatchery of Brackishwater Station, BFRI, Paikgacha, Khulna. The study was conducted during 2021-2022 fiscal year. Microalgae samples were collected from the nearby Sibsha river, adjacent shrimp *ghers* and mangrove canals.

Collection, identification and isolation of microalgae
Microalgae sample was collected with 0.05 micron plankton net in monthly intervals. About 20L of water was passed through the net and the residuals accumulated in the bucket was stored in plastic bottles with iodine solution and carried to the laboratory. A sub-sample of the microalgae was placed under compound microscope and available micro-algae were identified with the help of manual and checklists (Sieburth *et al*. 1978, Guiry and Guiry 2020) on coastal and marine microalgae.

Culture of microalgae

Spirulina platensis culture was done using three different media *viz*., (T_1) kosaric media (KM), (T_2) the Zarrouk media (ZM) and (T_3) locally developed media, named Bangla media (BM). Preparation of ZM and KM was done according to the media chart (Guillard 1975). Whereas, BM was used to reduce the production cost. *Nannochloropsis oculata* was cultured in outdoor conditions using the F2 media (Guillard 1975) supplemented with inorganic fertilizer ammonium sulfate (T_4). Each of the culture media had 3 set of replications in 300 L glass fiber tanks and the duration of culture was 6-12 days. Culture of live feed (microalgae) was performed following the standard protocol of microalgae culture by inoculating 0.5×10^6 cells/ml and the trial was repeated for thrice. A sub-sample of green algae from each culture treatment was collected with a sterile syringe every day and examined under microscope after serial dilution to count the cell density. Partial harvesting of green was done after 6-7 days as the density reached the peak.

Culture of live feed rotifer

Rotifer (*Brackionus plicatilis*) culture was done with three feeding regimens *viz*., with live Spirulina (T_1), with live *Nannochloropsis* (T_2) and with Baker's yeast (T_3) during November-December, 2021. Each of the treatment had three replications and 1000 L glass fiber tank was used as culture vessel. Water salinity was set at 25 ppt and inoculums density of rotifer was 15-20 ind./ml. Duration of each of the culture was 7-10 days and repeated for thrice. A sub-sample of rotifer from each culture treatment was collected with a sterile plastic dropper at every two-days intervals and examined under microscope to count the density. Harvesting of the rotifer was done at the end of culture with 50 μm plankton net to estimate the growth for enrichment.

Enrichment of rotifer cultured under different protocols

Rotifer cultured with different feeding regimen often lack of sufficient nutrients to support the growth of marine fish and crustacean larvae. Thus the rotifers needed to be fortified before feeding to the larvae. Rotifer culture and fortified trial was conducted with four treatments *viz*., with Spirulina powder (T_1), with *Nannochloropsis* (T_2), with baker's yeast (T_3) and with crude marine fish oil (T_4) called SELCO. The experiment was conducted in 50 L plastic buckets and three replications were assigned for each treatment. Density of rotifer was 500 ind/ml and the enrichment duration was 12 hours. Rotifer was harvested with 50 micron harvest net, dried and packed separately with labeling for proximate composition analysis.

Proximate composition analysis of live feed (microalgae and rotifer)

The proximate composition of two microalgae species (Spirulina and *Nannochloropsis*) and rotifer fortified with different media was analyzed from the Bangladesh Council of

Scientific and Industrial Research (BCSIR), Dhaka as according to the standard protocol of (Brown *et al.* 1989).

Data Analysis
All the data obtained was computed in MS Excel according to the trials. Data analysis was performed using MS excel, SPSS and presented with tabular and graphical forms.
Results

Identification of microalgae collected from coastal watershed
A total of 16 species of microalgae was identified from the adjacent watershed included shrimp *ghers*, rivers and mangrove canals. List of available microalgae has been stipulated in Figure 1. Among the identified microalgae, *Nannochloropsis oculata* and *Spirulina platensis* was found potential for crustacean and fish larvae rearing in coastal hatcheries. In addition, a single zooplankton (*Brachionus plicatilies*) was also identified as prospective for the fast feeding to the larvae of crustacean and fishes.

Gymnodinium spp	*Spirulina platensis*	*Coscino discus*
Chaetoceros spp	*Spirogyra spp*	*Planktoniella spp*
Thalassionema spp	*Thalassiosira spp*	*Nannochloropsis oculata*
Isochrysis spp	*Skeletonema costatum*	*Tetraselmis spp*
Cylindrotheca spp	*Ditylum spp*	*Ditylum sol*
Biddulphia mobiliensis		
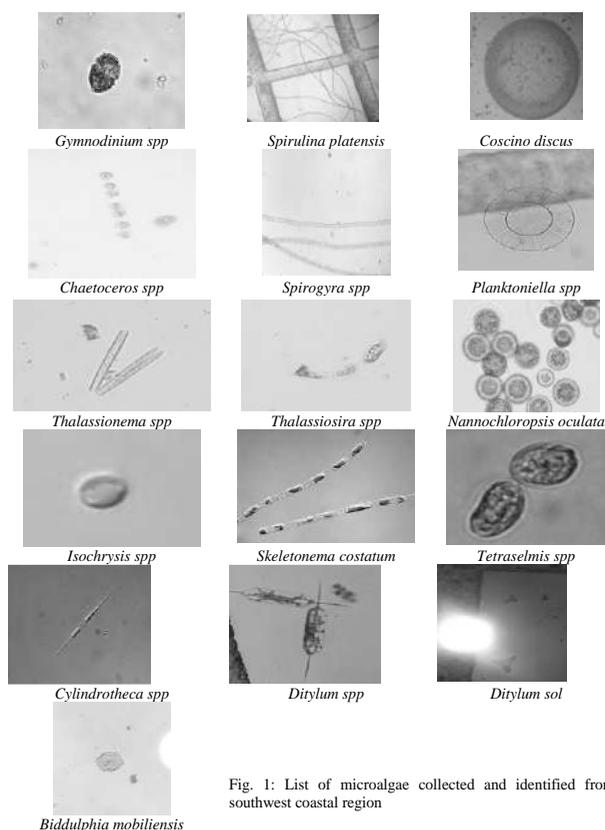
Fig. 1: List of microalgae collected and identified from southwest coastal region

Growth of green live feed (*N. oculata* and *S. platensis*)

Water temperature of 24 to 25 °C, pH 7.5 to 8.5, salinity 30 ppt, and light intensity 2000-4000 lx was maintained for culture of *N. oculata* and *S. platensis*. The microalgae (*N. oculata*) started cell division immediately after inoculation; lag phase took 2-3 days and reached the peak on the 7th day of culture. The exponential phase lasted for 5-7 days and then collapsed sharply (Fig. 2). In outdoor conditions the highest growth of *N. oculata* was $59.70 \pm 6.50 \times 10^6$ cells/ml in the second trial and lowest $35.67 \pm 7.88 \times 10^6$ cells/ml was in 1st trial with an average cell density of $46.50 \pm 11.97 \times 10^6$ cells/ml (Table 1).
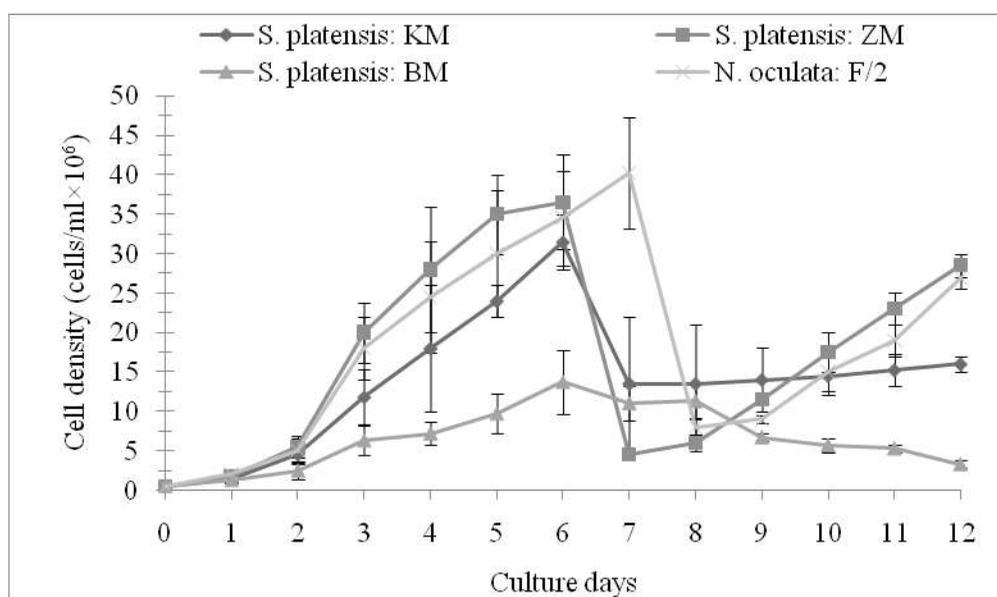
Fig. 2. Growth pattern of live feed (*N. oculata* and *S. platensis*) under different culture media.

Spirulina always showed higher growth in Zarrouk's media (ZM) than Kosaric media (KM), and Bangla media (BM) showed lower growth during 12 days culture period (Fig. 2). Cell density of Spirulina doubled at four days and highest density was on the 6th day for all type of media. Partial harvesting was done on 7th day and supplemented with media for regeneration (Fig. 2). Among the three successive trials Spirulina displayed better growth in the 2nd trial under all type of media. Average growth of Spirulina was $28.59 \pm 3.93 \times 10^6$ cells/ml, $32.59 \pm 3.04 \times 10^6$ cells/ml and $19.92 \pm 1.81 \times 10^6$ cells/ml in KM, ZM and BM, respectively (Table 1). However, average growth of *N. oculata* ($46.50 \pm 11.97 \times 106$) was higher than *S. platensis* ($32.59 \pm 3.04 \times 10^6$) (Table 1).

Table 1. Growth (Mean±SD×10^6) of *N. oculata* and *S. Platensis* under different media

Nos. of trials	*N. oculata* (cells/l)	*S. platensis* (cells/l)		
	f/2 media	Kosaric media	Zarrouk media	Bangla media
Trial-1 (N=3)	35.67±7.88	25.60±5.23	32.75±3.74	18.02±1.30
Trial-2 (N=3)	59.70±6.50	32.37±1.23	34.68±2.94	21.30±0.99
Trial-3 (N=3)	44.12±4.27	27.80±0.70	30.35±2.19	20.45±1.48
Average (N=9)	46.50±11.97	28.59±3.93	32.59±3.04	19.92±1.81

Growth performance of rotifer (B. plicatilies)

This experiment showed that as the culture period increased (in days), the number of rotifers (ind./ml) gradually increased. The highest population growth of rotifer (114 ind./ml) was observed at the end of the culture period (on the 8th day) fed with live *Nannochloropsis*, which was considerably higher compared to Baker's yeast (84 ind./ml) and Spirulina powder (78 ind./ml) (Fig. 3). On the other hand, Baker's yeast fed group had knowingly higher population growth compared to the spirulina powder fed group.
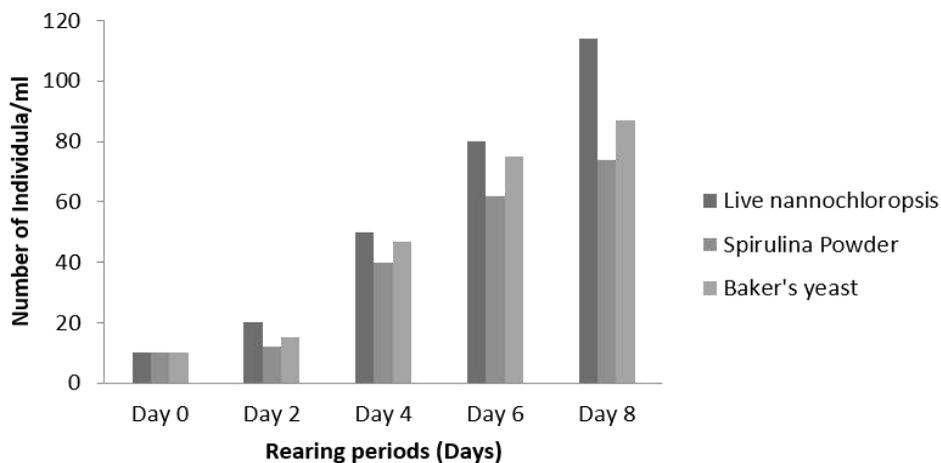
Fig. 3. Growth performance of rotifer under different feeding regime.

Proximate composition of live feed (microalgae)

The proximate composition of two species of micro-algae is computed and compared in Table 2. Although there are marked differences in the compositions of the micro-algal classes and species, protein is always the major organic constituent, followed usually by lipid and then by carbohydrate. The level of protein, lipid, and carbohydrate in *S. platensis* was 57.44±0.32%, 12.2±0.56% and 6.2%, respectively (Table 2). Whereas, *N.*

oculata contained the protein, lipid and carbohydrate levels of 53.75±0.32%, 13.0±0.01% and 7.2±05%, respectively. *S. platensis* was rich in protein, whereas *N. oculata* was rich in lipid and carbohydrate content.

Table 2. Proximate composition (%) of live feed (microalgae) cultured under outdoor conditions

Parameters	*S. platensis*	*N. oculata*
Dry weight	15.2±0.41	16.3±0.025
Calories (100g dry wt. algae)		443±0.20
Protein	57.44±0.32	53.75±0.32
Lipid	12.2±0.56	13.0±0.01
Carbohydrates	6.2±0.91	7.2±05
Ash	7.2±0.31	8.0±0.21
Vitamin C	0.89±0.11	0.87±0.51
Chlorophyll-A	0.87±0.06	0.88±0.71

Proximate composition of live feed (rotifer, Brachionus plicatilies)

The nutritive value of rotifer (*Brachionus* sp.) was evaluated after enrichment with different media. The highest level of protein 51.2±0.31% was found in rotifer enriched with crude fish oil followed by 41.3±0.76%, 38.4±0.67 % and 35.5±0.91% for rotifer enriched with *S. platensis, N. oculata* and Baker's yeast, respectively (Table 3). On the other hand, maximum lipid content 25.5±0.32% was found in rotifer enriched with fish oil followed by 20.2±0.76%, 18.9±0.87% and 18.1±0.24% for rotifer enriched with *N. oculata, S. platensis* and Baker's yeast, respectively (Table 3). However, rotifers fortified with crude fish oil provided better levels of protein and lipids than other three media.

Table 3. Proximate composition (%) of live feed cultured and enriched with different media

Parameters	*S platensis*	*N. oculata*	Baker's yeast	Fish oil
Protein	41.3±0.76	38.4±0.67	35.5±0.91	51.2±0.31
Lipid	18.9±0.87	20.2±0.76	18.1±0.24	25.5±0.32
Carbohydrate	21.5±0.81	26.5±0.12	23.5±0.23	12.1±0.25
Ash	5.1±0.45	4.9±0.13	6.9±0.41	3.9±0.40
Dry matter	4.3±0.14	3.8±0.58	4.2±0.56	3.9±0.50
Moisture	8.9±0.17	6.2±0.29	11.8±0.18	3. 4±0.11

Discussion

Development of artificial diets for brackishwater and marine larvae rearing is the great challenge as specific food requirements in their larval development and the only

suitable feeds are the live foods (Hoff and Snell 1989). Microalgae, the base of the food chain in aquatic ecosystems and initiating of carbon production, many zooplanktons including fish larvae require the highly nutritious microalgae for their population growth. Fish and crustacean larvae, which are predator upon other prey zooplankton likewise, must receive their required nutrition from these prey species or else they will not develop successfully to metamorphosis (Hoff and Snell 1989, Braley 1994). *Nannochloropsis* and *Spirulina* are the most popular genera of microalgae cultured and used for aquaculture (Liao and Huang 1970, Laing and Millican 1986).

A number of fundamental steps including water, light and media have been linked with successful microalgae culture (Thompson 2009). The most important parameters affecting microalgae growth are nutrient levels and value, pH, aeration mixing, salinity and temperature (Coutteau 1996). The range of temperature: 20-30°C; light intensity: 2500-8000 lux and salinity: 10-36 ppt is generally used to culture *N. oculata* (Hoff and Snell 1999). In this experiment, two species of microalgae viz., *N. oculata* and *S. platensis* has been cultured and the ambient environment maintained has been strongly supported by Hoff and Snell (1999). Green algae cultivated in this experiment took 3 days in stationary phase, 6-7 days in the exponential phase and reached to the peak on 6th and 7th days for *S. platensis* and *N. oculata*, respectively. However, the duration of exponential phase is influenced by the nutrient levels and quality, source of light and intensity, carbon dioxide levels, type of culture vessels, and other physical and chemical parameters (Hoff and Snell 1999).

In Singapore, Lim (1991) used initial inoculums of 3–4x10^{6} cells/ml of *N. oculata* cultured *in* 3 L and 20 L polythene bags. He obtained final density of 2.00–2.50x10^{7} cells/ml within seven days in 3 L bags but it was 1.80–2.00x10^{7} cells/ml within five days in 20 L bags. Growth of *N. oculata* seemed much better under indoor conditions where 24 hours artificial light was provided than the outdoor culture depending on day-night lighting system (Lim 1991). In this study, average final density of *N. oculata* was 46.50±11.97x10^{6} cells/ml in F/2 media within 7 days and it was 19.92±1.81 x 10^{6} cells/ml to 32.59±3.04 x 10^{6} cells/ml for *S. platensis* under different media within 6 days. The final density obtained in this study seemed lower than the observation of Lim (1991). This might have happened due to lower inoculums density in this study and differences in culture vessels and conditions. However, the exponential phase seemed in line with the observation of Lim (1991). Microalgae culture crashes are associated with the factors included exhaustion of nutrient(s), low oxygen levels, high temperatures, fluctuations in pH, and/or contamination; and the success of phytoplankton culture depends on maintaining the cultures in the exponential phase (Coutteau 1996).

Phytoplankton culture crashes are related to the local weather and seasons due to a lack of adequate sun light and contamination (Fulks and Main 1991, Martin *et al.* 2012).

Protein is an essential component of a diet. Hence, there is an urgent need to find a new source of protein independent on agriculture or fish meal. The best potential option is in microbial protein or single cell protein (SCP), SCP are characterized by fast growth rate, high protein content (43-85%) compared to field crops, require less water, land and independent of climate, grow on wastewater can be genetically modified for desirable characteristics such as amino acid composition and temperature tolerance (Perkebunan 2001). Spirulina has been used as a complementary dietary ingredient in feed for fish, shrimp and poultry and increasingly as a protein and vitamin supplement to aqua feeds (Ciferri and Tinoni 1985). In our study, spirulina contains 57.44% crude protein, 6.2% carbohydrate and 12.2% lipid. *N. oculata* contains 53.75% protein, 13.0% lipid and 7.2% carbohydrate. It was similar to Chung *et al*. (1978) who found 45 to 61% crude protein in spirulina green algae. Devi *et al*. (1981) reported 50-65% of protein in green algae, of which nearly 9.9 percent was non-protein nitrogen. The high level of lipid in *N. oculata* and *S. platensis* is rich with the essential fatty acid of eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) have made them suitable green water/live feeds for increasing the fatty acid content of rotifers (Dhert 1996).

Marine Rotifers and *Artemia* are well known for marine larvae rearing for its special features of rapid growth, slow motility and smaller size and easy to culture (Snell and Carrillo 1984). It is known as 'first food' of larvae for marine hatchery operation. The highest mean rotifers were observed in the live *Nannochloropsis* fed group (114 ind./ml) at the end of the rearing period, which was significantly higher than that of the Baker's yeast and powdered spirulina fed groups. The growth of rotifer seemed lower than that of Islam *et al.* (2017), might be due to lower temperature (22-24 ºC) during the culture period. Many studies have shown that the production of rotifers is greatly influenced by their food (Lubzens 1987, Lubzens 2001). The growth of many rotifer species is mostly influenced by the types of food they ingest and their feeding rations (Sarma and Rao 1987). Moreover, the size and shape of food particles, mobility, digestibility, and nutritional composition are also responsible for the growth of rotifers. Our result was similar to that of Das *et al.* (2021), who found that rotifers fed fish oil and live *Nannochloropsis* sp. showed significantly better population growth and an instantaneous growth rate compared to Baker's yeast and powder spirulina. FAO (1996) suggested growing rotifers (*B. plicatilis*) at the temperature above 26 °C and water salinity of 25 ppt for optimum growth. Rotifers (*B. plicatilis*) retain the nutrition from their food for a short period only. Thus, they must be consumed by the predator larvae soon after the rotifer has eaten microalgae or the predator species will have nutrient-

depleted food (Hoff and Snell 1989, Braley 1994). Rotifers that grown in baker's yeast or less microalgae are enriched with marine fish oil emulsion (SELCO, INVE) to enhance their nutritional content and duration of enrichment can reach 6-12 hours before feeding to larvae (Islam *et al.* 2017). Thus the nutrient content of rotifers fed/enriched with crude fish oil showed better levels of protein (51.2%) and lipids (25/5%) than other groups in this study. Meanwhile, Dhert (1996) observed enhanced level of EPA and DHA in rotifers fortified with marine fish oil emulsions.

Microalgae are important source of nutrition and widely used in the aquaculture of other organisms, either directly or as an added source of basic nutrients. In the brackishwater fields live feed, micro-algae and rotifers are essential sources of larvae feed and crucial sources of their survival and growth. For this reasons, live feed identification is an important factor in determining the culture mechanisms of brackishwater fin fishes and crustacean and mollusca in coastal fisheries management. However, green live feed of *N. oculata* could be cultivated by using the F/2 media and *S. platensis* with either kosaric media (KM) or Zarrouk media (ZM) to maximize the production. Rotifer culture could be done with live *N. oculata*, spirulina powder or baker's yeast. It is strongly suggested to fortify the rotifer with marine fish oil to enhance the nutritive value before feeding the larvae.

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Fish biodiversity, threats and current conservation status of Gumani river at Chalan *beel*, Bangladesh

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Abstract

The study was conducted from July 2022 to June 2023 to assess the biodiversity of fishes in the River Gumani, Chalan beel. A total of 69 fish species belonging to 10 orders and 27 families were documented. Cypriniformes was found to be the most dominant order consisting 35% of the total fish population. Eighteen threatened fish species were recorded from the River Gumani, of which 9 species (13%) were found as Vulnerable (VU), 8 species (11%) as Endangered (EN), and 1 species (1%) as Critically Endangered (CR). The present study mainly focuses on the fish biodiversity of the River Gumani and their current in respect of IUCN Red List status in Bangladesh. Appropriate recommendations of the open water management need to be followed for policy formulation and conservation of fisheries biodiversity.

Keywords: Gumani River, Fish Biodiversity, Conservation, Chalan Beel

Introduction

Chalan *beel* is an important water resource in the northwestern region of Bangladesh. This *beel* is also considered as the largest *beel* in the country. Historically Chalan *beel* is spread across 18 Upazila of six districts of Bangladesh. A total of 93 minor *beel* and floodplains adjacent to *beels*, 12,817 ponds, 214 Kua, and about 21 rivers are included in the Chalan *beel*. The total area of this beel is about 37,500 hectares during the monsoon and comes down to about 5,229 hectares during the dry season. The depth of water is about 2-2.5 meters in most areas of Chalan *beel* (Hossain *et al.* 2009). Chalan Beel is a principal reservoir of biological diversity in the Northeast region. It plays an important role in maintaining the environmental balance of the surrounding vast area.

Currently, the Chalan *beel* area is under threat and getting seriously depleted day by day. Numerous 'Kua' and 'Brush shelters' have been constructed in the Chalan *beel* area under private ownership. These 'Kua' and 'Brush shelters' are put into operation when

the monsoon water recedes, as a result of which fish species get trapped. Later, by drying the well, all kinds of larvae, prawns, and brood fishes are caught by wrapping the Brush shelter's net. Besides, the biodiversity of wetlands is under constant threat due to environmental climate change. Along with these trends in the current *beel*, anthropogenic activities are also responsible for *beel* contraction. Because, that *beel*, which is important in fish production, has not been declared an Ecological Critical Area (ECA) till now. A comprehensive study on Chalan *beel* is now timely and much needed. However, in the current situation, the present research provides a brief description of the conservation status of fish species existing in the important Gumani river, which will be useful in future research and management initiatives related to the Chalan *beel*.

Materials and Methods

Study area and period
The study was carried out in Gumani river (Rautara Sluwich Gate 24°08'17.6"N 89°33'30.4"E) at Chalan *beel* area. Data were collected from previously selected sampling points of the *beel* area on monthly basis. The study was conducted for a year during July 2022 to June 2023.

Data collection framework
Data and information were collected personally through field visit observation from sample sites. Information on fish species was collected through interviews with commercial fishing boat owners, retailers, fish traders, local people, fishermen, riverside settlers, and other key informants in the sampling area. Focus group discussion (FGD) was also conducted in the fish landing center, fish market, and fisher's village of those selected sampling sites. Visits were made at least once a month during the study period.

a) **Fish specimen collection and identification:** Fish species were sampled from fishermen's catches and from various fish landing points as well as fish markets. Morphometric and meristic traits were evaluated and fish samples identified according to Rahman (2005), Talwar *et al.* (1991); the valid scientific names of the identified fish species were ensured by checking catalogue of life Roskov *et al.* (2016).

b) **Determination of conservation status:** Local conservation status was confirmed using IUCN Bangladesh (2015).

c) **Order-wise fish composition:** Identified fish species were categorized according to their taxonomic rank.

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Data analysis
Collected data was analyzed by computer software Microsoft Excel 2010.

Results

Fish diversity
A total 69 species under 10 orders and 26 families were recorded from the sampling stations. List of existing fish species with their taxonomic position (order and family name), scientific name, local name, group, and their IUCN conservation status in Bangladesh aspects are presented in Table 1.

Order wise percentage of Gumani river fish species
Cypriniformes was found to be the most dominant order consisting 35% of the total fish population followed by Siluriformes (26%), Perciformes (22%), Synbranchiformes (6%), Clupeiformes (3%), Osteoglossiformes (3%), Beloniformes (2%) and another 3 Order comprised 1% each of the total species (Fig. 1).
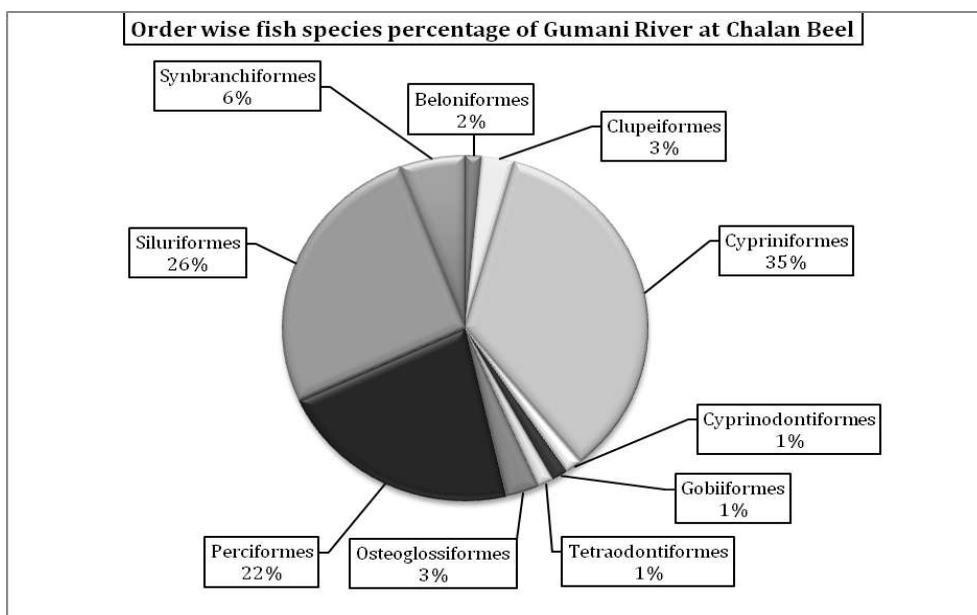
Fig. 1. Order wise fish species percentage of Gumani river at Chalan *beel*.

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IUCN Bangladesh conservation status of Gumani river fish species diversity

According to IUCN Bangladesh (2015), 64 indigenous freshwater fish species of Bangladesh have been declared as endangered fish species. 18 fish species have been identified in the Gumani river of Chalan beel which are threatened according IUCN Bangladesh (2015), which covers 28% of IUCN Bangladesh (2015). Out of 69 fish species, 38 species (56%) are Least Concern (LC), 13 species (19%) Near Threatened (NT), 9 species (13%) Vulnerable (VU), 08 species (11%) Endangered (EN), and 1 species (1%) was found as Critically Endangered (CR). No fish were found in the Data Deficient category (Fig. 2).
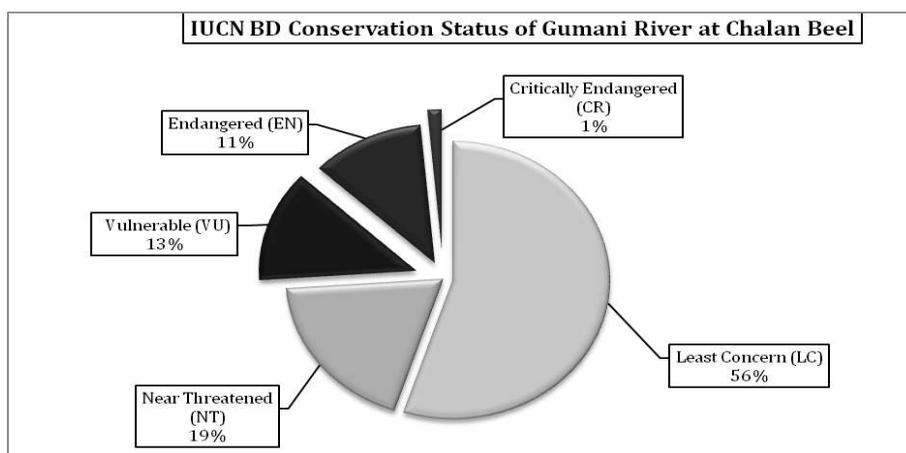
Fig. 2. IUCN conservation status of Gumani river at Chalan *beel*.

Discussion

A number of authors from Bangladesh at various times have reported the number and conservation status of various fish species in Chalan *beel*.

A study by Ahashan *et al.* (2008) observed 19 species of fish of commercial importance while looking at the fish marketing system of the Chalan *beel* area. A market survey conducted from July to December 2008 observed that 10 species were in high abundance and 9 species were in low out of a total of 19 species. Samad *et al.* (2009) identified 26 fish species used for fish drying from September 2006 to February 2007 in Atrai (Nawgaon), Singra (Natore), Bhangura (Pabna) and Tarash (Sirajganj) regions of Chalan *beel*. Seventeen (17) species were collected from Atrai, eighteen (18) from Singra, twenty (20) from Taras and twenty-three (23) from Bhangura, Pabna. Galib *et al.* (2009) identified 81 species of fish belonging to 12 orders and 27 fish families in Chalan

beel areas [Atrai Upazila (Naogaon); Natore Sadar, Singra, Baraigram, Gurudaspur (Natore); Chatmohor, Bhangura (Pabna); Nandigram (Bogra); and Tarash (Sirajganj) Upazila] during the period from September 2006 to August 2007. There are 72 indigenous and 9 exotic species of fish among them. The top three most populous fish orders were Cypriniformes (30 species, three families), Siluriformes (22 species, eight families), and Perciformes (12 species, seven families). Hossain *et al.* (2009) conducted fieldwork in 2005 and 2006 in 10 Upazila including three Rivers in the Chalan *beel* area (Singra, Gurdaspur, Baraigram, Chatmohar, Bhangura, Faridpur, Shahjadpur, Ullapara, Tarash and Raiganj, in three districts (Natore, Pabna, Sirajganj). Recorded data on 114 species of fish belonging to 29 families. Four most abundant fish species (*Puntius sophore, Puntius ticto, Chanda nama, Perambasis ranga, Mystus vittatus* in Gumani river, *Gudusia chapra* in Baral and Katagang Rivers) were observed. In the author's study, 10 species are feared to be threatened in the future. Indicates that the following species may be threatened. Besides, a decreasing trend was observed in the variability of five species each in the Gumani and Baral rivers and six species in the Katagang river.

Kostori *et al.* (2011) found 82 SIS during the period from September 2010 to August 2011 in Chalan *beel* (Natoresadar, Singra, Baraigram and Gurudaspur of Natore district, Chatmohor and Bhangura of Pabna district, Tarash of Sirajganj district and Atrai of Noagaon district). Among the 82 SIS arranged in 10 orders and 22 families, Cypriniformes (42.68%) is the most dominant order. Jahan *et al.* (2016) observed the presence of 53 species (including exotic species) belonging to 7 Orders, and 17 families in the fishing gear of Natore district (Natore Sadar, Singra, Gurudaspur and Baraigram) from July to December 2012. Siddique *et al.* (2016) recorded 78 species of fish belonging to 10 orders and 26 families at Chalan *beel* (Singra, Chatmohor, Tarash and Atrai) from January 2015 to December 2015. There are 69 domestic and nine (9) exotic species of fish.

Khanom *et al.* (2022) identified 42 species of fish belonging to 07 orders and 18 fish families during the period from January-October 2020 to January-October 2021 in Chalan *beel*. There are 40 indigenous and two foreign species of fish among them. Chowdhury *et al.* (2022) recorded 88 species of fish belonging to 9 orders and 24 families from Chalan *beel*. Among the fish species collected during the period from July 2020 to December 2020, there are 78 indigenous and 10 exotic fish species. Hannan *et al.* (2023) recorded a total of 62 fish species in Chalan *beel* (Singra and Natore Sadar Upazila of Natore district), including 51 native fish species and 11 exotic species. Fishes of 12 orders were recorded. 8 species have been identified as Threatened (*Labeo bata, Mylopharyngodon piceus, Mystus aor, Eutropiichthys vacha, Rita rita, Nandus nandus, Notopterus chitala, Tetraodon patoca*).

Ahashan *et al.* (2008), Samad *et al.* (2009), Galib *et al.* (2009), Hossain *et al.* (2009), Kostori *et al.* (2011), Jahan *et al.* (2016), Siddique *et al.* (2016), Khanom *et al.* (2022), Chowdhury *et al.* (2022), and Hannan *et al.* (2023), among studies completed between 2008 and 2023, Hossain *et al.* (2009) is the only author who analyzed the data of 3 Rivers. One of the 3 Rivers is the Gumani river. In the author's study, 10 species are threatened in the future. Five species of fish have been observed to decrease trend in the Gumani river. The fish diversity of the Gumani river is discussed in this article. Currently 18 species of fish are identified as threatened in Gumani river. The present study focuses mainly on the fish biodiversity of the Gumani river in Chalan *beel* and documentation of their current conservation status in Bangladesh. Overall, research and management initiatives on Chalan *beel* have been taken up very little. A comprehensive study on movement *beels* is timely and much needed.

The following recommendations can be followed for policy formulation, implementation and conservation of fish biodiversity of Chalan *beel*:

- Prohibition of unregulated and indiscriminate fishing in *beel*; also prohibiting the use of destructively designed nets.
- Prohibition on the construction of 'Kua' and 'Brush shelters' in private ownership in the Chalan *beel* area.
- Fishing should be prohibited during the spawning season to protect the larvae and fry of naturally spawning fish.
- Identify and establish new and permanent fish sanctuaries (breeding and nursery areas) in Chalan *beel* through research.
- New and old fish sanctuaries should be regularly maintained to ensure their safety.
- The establishment of government fish landing centers in the *beel* area and a fisher-friendly trade system should be implemented.
- Policies should be formulated considering the environment, surroundings, and anthropology of the rivers and land-*beel* areas (6-7 months submerged) within the Chalan *beel*.
- Declaring this *beel* as an 'Ecological Critical Area (ECA)' is important for fish production.

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Table 1. List of fish species collected from the River Gumani at Chalan *beel*

Order	Family	Scientific Name	Local Name	Group Name	IUCN Status (BD)*
Beloniformes	Belonidae	*Xenentodon cancila*	Kakila	Gar	LC
Clupeiformes	Clupeidae	*Gudusia chapra*	Chapila	Shad	VU
		Corica soborna	Kachki	Shad	LC
Cypriniformes	Cyprinidae	*Labeo calbasu*	Kalibaus	Carp	LC
		Labeo rohita	Rui	Carp	LC
		Cirrhinus cirrhosis	Mrigal	Carp	NT
		Cirrhinus reba	Bhagna	Carp	NT
		Gibelion catla	Katla	Carp	LC
		Labeo bata	Bata	Barb & Minnows	LC
		Puntius sarana	Sarpunti	Barb & Minnows	NT
		Puntius chola	Chalapunti	Barb & Minnows	LC
		Puntius sophore	Punti	Barb& Minnows	LC
		Barbonymus gonionotus	Puti	Barb & Minnows	LC
		Esomus danricus	darkina	Barb & Minnows	LC
		Pethia ticto	Puti	Barb & Minnows	VU
		Pethia conconius	Darkina	Barb & Minnows	LC
		Aspidoparia morar	Pialy	Barb & Minnows	VU
		Amblypharyngodon mola	Mola	Barb & Minnows	LC
		Salmostoma bachila	Narkeli chela	Barb & Minnows	LC
		Osteobrama cotio	Dhela	Minnows or carps	NT
		Salmophasiaphulo	Fulchela	Barb & Minnows	NT
	Danionidae	*Salmostoma acinaces*	Chela	Barb & Minnows	LC
	Balitoridae	*Acanthocobitis botia*	Balachata	Loach	LC
	Cobitidae	*Lipidocephalichthys guntea*	Puia	Loach	LC
		Botia dario	Rani	Loach	EN

		Canthophrys gongota	Ghora Gutum	Loach	NT
		Botia lohachata	Bou/Rani	Loach	EN
Cyprinodontiformes	Aplocheilidae	*Aplocheilus panchax*	Kanpona	Killi/Rice	LC
Gobiiformes	Gobiidae	*Glossogobius giuris*	Bele	Mudskipper	LC
Tetraodontiformes	Tetraodontidae	*Tetraodon cutcutia*	Potka	blowfish/Puffers	LC
Osteoglossiformes	Notopteridae	*Notopterus notopterus*	Foli	Feather backs	VU
		Chitala chitala	Chitol	Feather backs	EN
Perciformes	Anabantidae	*Anabas testudineus*	Koi	Perch	LC
	Badidae	*Badis badis*	Kholisa	Perch	NT
	Nandidae	*Nandus nandus*	Vheda	Perch	NT
		Nandus meni	Meni	Perch	NT
	Channidae	*Channa gachua*	Chang	Snakehead	LC
		Channa punctata	Taki	Snakehead	LC
		Channa striata	Shol	Snakehead	LC
		Channa orientalis	Chang	Snakehead	LC
		Channa marulius	Gojar	Snakehead	EN
	Osphronemidae	*Trichogaster faciata*	Kholisa	Perch	LC
		Trichogaster lalius	Kholisa	Perch	LC
		Trichogaster chuna	Kholisa	Perch	LC
	Ambassidae	*Pseudambassis baculis*	Chanda	Perch	NT
		Parambassis ranga	Rangachanda	Perch	LC
		Chanda nama	Nama chanda	Perch	LC
Siluriformes	Siluridae	*Wallago attu*	Boal	Catfish	VU
	Bagride	*Sperata seenghala*	Guizza air	Catfish	VU
		Sperata aor	Air	Catfish	VU
		Rita rita	Ritha	Catfish	EN
		Hemibagrus menoda	Ghagla	Catfish	NT

		Mystus tengra	Tengra	Catfish	LC
		Mystus bleekeri	Gulsa Tengra	Catfish	LC
		Mystus vittatus	Tengra	Catfish	LC
		Mystus cavasius	GolsaTengra	Catfish	NT
	Heteropneustidae	*Heteropneustes fossillis*	Shing	Catfish	LC
	Schilbeidae	*Neotropius atherinoides*	Batasi	Catfish	LC
	Claridae	*Clarias batrachus*	Magur	Catfish	LC
	Pangasiidae	*Pangasius pangasius*	Desi Pangus	Catfish	EN
	Schilbeidae	*Clupisoma garua*	Ghaura	Catfish	EN
		Eutropiichthys vacha	Bacha	Catfish	LC
	Sisoridae	*Bagarius bagarius*	Baghair	Catfish	CR
		Gagata cenia	Kauwa Tengra	Catfish	LC
		Glyptothorax telchitta	Telchitta	Catfish	VU
Synbranchiformes	Synbranchidae	*Monopterus cuchia*	Kuchia	Eel	VU
	Mastacembelidae	*Mastacembelus armatus*	Baim	Eel	EN
		Mastacembelus pancalus	Guchi	Eel	LC
		Mastacembelus aculeatus	Guchi Baim	Eel	NT

* Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically Endangered (CR), Data Deficient (DD).

Enhancing spawning success of *Tenualosa ilisha* (Hamilton, 1822) in Bangladesh through implementing fishing ban: A practical approach

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Abstract

The study aimed to rigorously assess the impact of fishing ban imposed during 12 October – 2 November, 2023 for Hilsa, *Tenualosa ilisha*, on its breeding success covering an area of approximately 7,000 sq km in Meghna River basin. The percentage of spent Hilsa (locally termed as *pite*) and actively spawning Hilsa (Oozing) were monitored following a standardized protocol. Among all the Hilsa captured within and surrounding the spawning grounds, males constituted 35-45%, while females constituted 85-95% of the total catch, indicating a male-to-female sex ratio of 1:2.3. The breeding success rate in 2023 recorded a significant 52.4%, means that 52.4% of Hilsa successfully participated in the breeding process, marking an impressive 104.8% increase compared to the base year 2001-02. The estimated quantity of fertilized eggs in 2023 amounted to be 811,711 kg. The heightened production of Hilsa eggs and Jatka underscored a positive impact of the twenty-two-days fishing ban. Moreover, percentages of gravid and actively spawning Hilsa were notably higher compared to previous years. The enforcement of a complete fishing ban led to a remarkable increase in the proportion of female Hilsa in the breeding areas reached 88% which was impressive. In summary, the twenty-two-days fishing ban during the peak breeding period proved to be highly effective for ensuring the successful spawning of Hilsa, highlighting its significant role in Hilsa conservation efforts.
Keywords: *Tenualosa ilisha*, Hilsa, Fishing ban, spawning success

Introduction

An anadromous shad, Hilsa (*Tenualosa ilisha*) follows a distinctive life cycle wherein it breeds in the upstream rivers, with the larvae hatching from freely floating eggs. The immature juveniles grow within river channels before migrating to the sea for feeding and further development, eventually returning to the rivers as mature gravid Hilsa to complete the life cycle. It's also known for its high fecundity, can produce up to 2 million eggs (Rahman *et al.* 2017). While Hilsa spawns throughout the year, the primary spawning season occurs during the Bengali month of Ashwin-Kartik (September-October), coinciding with the full moon phase. The extensive capture of mature gravid

Hilsa during this peak spawning season in major spawning grounds, along with the interception of juvenile Hilsa (jatka) during their seaward migration in major rivers, constitutes significant factors contributing to the decline of the Hilsa fishery in the country.

Given the pivotal role of Hilsa in nutrition, employment, and the economy, the formulation of the Hilsa Fishery Management Action Plan (HFMAP) was imperative to foster the development, management, and conservation of Hilsa. Restrictions have been imposed in accordance with HFMAP guidelines to ensure the safe and successful spawning of Hilsa. These restrictions include a 22-day ban period, from 12th October to 02nd November in 2023, and included a new moon and full moon day (Bengali month Ashwin,1429), spanning four days before the full moon of the Bengali month of Ashwin (1427 Bengali year), the full moon day, and 17 days thereafter, from October 14th to November 4th, 2020. During this period, all commercial trawlers are prohibited from fishing in the sea, coastal areas, and rivers under the Marine Fisheries Ordinance 1983, Section 55, Sub-section 2 (D), facilitating safe migration and uninterrupted spawning of Hilsa. The implementation of the fishing ban was orchestrated by the Department of Fisheries (DoF), in collaboration with relevant stakeholders, including law enforcement agencies such as the Navy, Coast Guards, River Police, and Air Force. The Ministry of Fisheries and Livestock (MoFL) oversees the coordination of this ban program, guided by the research findings of the Bangladesh Fisheries Research Institute, Riverine Station.

Methodology

Study protocol

The Hilsa Research Team of BFRI, RS, Chandpur carried out all the investigations. Five research teams were formed to conduct a comprehensive investigation in the designated Hilsa spawning and sanctuary areas (Table 1). The nursery grounds were chosen as sampling sites because: (a) Hilsa shed their eggs in almost all major river systems across the country during the breeding season, but the spawning grounds grasp 80-90% of them; (b) Hilsa eggs and larvae begin drifting and shifting to upstream rivers shortly after spawning; and (c) Hilsa is a migratory fish that may travel a long distance after spawning and be captured there. As a result, a large sampling area was chosen to provide precise information on the percentage of spent, oozing, and gravid Hilsa.

Table 1. Six established sanctuary areas of *T. ilisha*

Sl. No.	Sanctuary area	Length	River	Ban period	District
1	Shatnol-Char Alexander	100 km	Lower Meghna estuary	March-April	Chandpur-Laxmipur
2	Char Ilisha-Char Pial	90 km	Stretch of Shahbajpur Channel, tributary of the Meghna river	March-April	Bhola
3	Bheduria-Char Rustam	100 km	Stretch of Tetulia river	March-April	Bhola-Patuakhali
4	Kalapara Golbunia-Confluence of Bay of Bengal and Andharmanik river	40 km	Andharmanik river	November-January	Patuakhali
5	Tarabunia-Vomkora	20 km	Lower Padma river	March-April	Shariatpur
6	Habinagar, Barisal Sadar-Hizla-Mehendigonj	82 Km	Meghna River	March-April	Barisal

Source: Bangladesh gazette 28 May, 2014 and 17 April, 2018, MoFL

Standard formula (Rahman et al. 2009 and Rahman et al. 2017)

The number of Hilsa rescued and total fertilized eggs as a result of the fishing enclosure were determined using the following formula:

No. of Hilsa saved due to fishing enclosure (TN) = Nos. of fishing boat*Nos. of haul/day*Nos. of fish captured/ Nos. of haul* Nos. of days----------------------------------(1)

Total fertilized eggs (Kg) = TN*FF*SF*EF/1000--(2)

Where, TN = Total No. of Hilsa excluded due to fishing enclosure;

EF = % of female fishes in the study areas;

SF = % of spent/oozing fish, and

EF = Average egg (g) per fish

Study area

The investigation focused on key Hilsa spawning grounds, nursery areas, and fish landing sites across 13 districts in Bangladesh (as outlined in Table 2 and Fig. 1). These locations were chosen due to their significance in hosting the peak spawning season for Hilsa, particularly along major river systems like the Padma and Meghna rivers. Additionally, samples were obtained from Chattogram and Cox's Bazar, where a large portion of marine Hilsa is typically gathered for sale.

Table 2. Selected sites for sampling

SL. No.	Sites	District	River/Sea
1.	Chandpur, Katakhali, Horinaghat, Haimchar & Charvoirobi	Chandpur	Meghna
2.	Hizla, Mehendiganj, Ulania	Barishal	Meghna
3.	Ramgoti, Char Alexandar	Laxmipur	Meghna
4.	Tanki Bazar, Chairman ghat, Hatia	Noakhali	Meghna estuary
5.	Dhalchar, Moulovirchar, Daulatkhan, Monpura	Bhola	Meghna estuary
6.	Kalirchar, Patharghata	Barguna	Meghna estuary
7.	Mohipur, Galachipa, Kalapara	Patuakhali	Andharmanik
8.	Tarabunia	Shariatpur	Padma
9.	Godagari	Rajshahi	Padma
10.	Mawa	Munshigonj	Padma
11.	Fishery Ghat	Chattagram	Sea
12.	BFDC Ghat	Cox's Bazar	Sea
13.	BFDC Ghat	Khulna	Sea
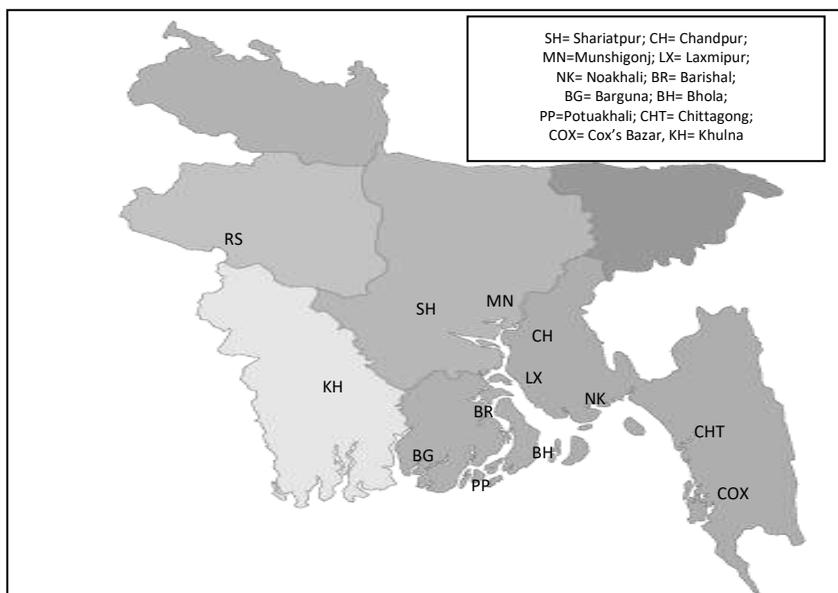
Fig. 1. Thirteen districts on the map from where data were collected during fishing ban.

Data collection
Three sequential time frames were selected for sampling and data acquisition by the Hilsa Research Team such as:

a) Before the ban period (07 days)
b) Whole ban period (22 days)
c) After the ban period (07 days)

Determination of size, sex and percent composition of gravid/berried Hilsa

The Hilsa samples collected were measured for their length (in centimeters) and weight (in grams) at various sampling locations, as detailed in Table 2. A wooden scale was used to measure length, while weight was measured using a digital balance. To determine the sex of the Hilsa fish, external observation and gentle pressure from the ventral squat to the anus were employed. If the fish emitted a liquid cream while being stripped, it was identified as male. Conversely, if the fish extruded eggs along with a light reddish substance, it was classified as a female Hilsa. Female Hilsa were also distinguished by their potbellied reddish appearance and larger anus. Fish that did not emit either milt or eggs were considered either immature (jatka) or partly mature. Generally, female Hilsa were observed to be longer in length compared to male Hilsa.

Identification of spent (pite 'in local language) and oozing (spawning) Hilsa

Spent Hilsa were identified by several external features such as a skinny appearance, a squeezed stomach, and an elongated slender body. When light pressure was applied to the abdomen and watery liquid or blood emerged along with alienated and malformed eggs, the fish was classified as spent Hilsa ('pite' in the local language). Spent Hilsa, upon being captured from the river, were often weak. Fishers typically keep spent Hilsa for their own household consumption and do not bring them to the market as they can reduce the market value of other fish. Oozing Hilsa were recognized by studying their egg-laying state, which involved ripe eggs spontaneously erupting without any pressure on the abdomen (Fig. 2).

Fig. 2. Pictorial views of oozing and spent Hilsa.

Gonadosomatic index of Hilsa

During the investigation, the sex of a minimum of 50 individual specimens was determined by making an abdominal incision and visually examining the gonads. The

gonads were carefully cleaned of all fat, connective tissue, and blood vessels, and their gonadal weight (GW) was measured to the nearest 0.001 grams. The gonadosomatic index (GSI) was then calculated using the formula: GSI = GW × 100/BW (Nikolsky 1963)

Determining the (%) of spent (pite) Hilsa

The quantity and proportion of spent Hilsa were assessed primarily by examining Hilsa caught using experimental nets in the breeding areas and monitoring commercial fish landing sites around these breeding grounds.

Calculation of fertilized eggs

During the fishing ban period, the approximate number of fertilized eggs released by Hilsa in the spawning grounds were estimated using the following formula:

The plausible amount of egg production due to the fishing ban was:

- Nos. of matured Hilsa captured in per unit effort = 26 (Two haul/day; = 52) (BFRI Investigation)
- Total nos. of fishing ban days = 22 days
- Approximate number of fishing boats = 15500 [Total area of spawning ground =7000 sq km, on an average 2 fishing boats are operated in per sq. km]
- Average percentage of matured female during the fishing ban = 78% (BFRI investigation)
- Average percentage of spent Hilsa during the fishing enclosure = 52.4% (BFRI investigation)
- Average weight of gonad of matured female = 112 g (BFRI investigation)
- One kilogram = 1000 g

Approximate egg production= {(52*22*15500*78/100) *(52.4/100*112)}/1000 = 811,711 kg

Calculation of jatka production

The number of fry and *jatka* production were calculated based on 50% hatching rate of fertilized eggs and 10% survival rate of hatched larvae.

Size, weight, age and CPUE calculation of Hilsa fry and jatka

Hilsa larvae were gathered from the spawning grounds and neighboring areas using a BFRI experimental fishing net crafted from glass nylon fiber. The length and weight of Hilsa larvae and jatka were measured using a measuring scale and a digital balance. External observations and available literature were consulted to estimate the approximate age of Hilsa fry and jatka. The quantity of jatka (in kg) caught in a 100-meter net per hour per boat was recorded as the catch per unit effort (CPUE).

Observations of other impacts of fishing ban

To assess the additional benefits of the 22-day fishing ban, larvae and juveniles of other fish species were collected from the spawning grounds and nearby areas using a BFRI experimental fishing net. This net was made of glass nylon fiber, with specific dimensions: mouth diameter of 15 feet, length from the mouth to the end of 25 feet, and a zero-sized loop. The fishing duration was set at 30 minutes. The percentage of other fish species' larvae and juveniles was calculated and compared to the Hilsa larvae, as well as to data from previous years. This comparative analysis helped to evaluate the impact of fishing closures on Hilsa spawning success and the enhancement of biodiversity among other fish species.

Data analysis

The data were compiled and analyzed using Microsoft Excel (ver. 2016). Additionally, graphical representations of the data were generated using Microsoft Excel to facilitate visualization and interpretation.

Result and Discussion

Size and sex ratio of Hilsa

In upstream areas, i.e. Chandpur, the majority of Hilsa measured less than 35 cm, constituting approximately 55% of the population. Conversely, in downstream areas like Bhola, over 75% of Hilsa were larger than 35 cm, with many being mature and gravid (Fig. 3). This observation aligns with Rahman *et al*. (2017), which investigated the impact of fishing bans during peak spawning times of Hilsa. Their findings showed a higher percentage of Hilsa in length groups greater than 40 cm in regions such as Monpura and Hatia. In addition to the spatial distribution of length-frequency (L-F) observed, there was also an apparent temporal shift in the length classes of Hilsa. Prior to the ban period, the 30-35 cm length class was predominant (Fig. 4). However, during and after the prohibition period, mature Hilsa (specifically egg-bearing females measuring over 40 cm) became predominant. Rahman *et al*. (2017) also noted a similar transformation in Hilsa length classes before, during, and after the spawning season. These findings suggest that a large school of mature and gravid Hilsa congregates at spawning grounds for breeding purposes, and implementing fishing bans appears to be an appropriate measure to promote their successful breeding.

The proportion of male and female Hilsa exhibited significant variation before and after the ban period, indicating an imbalance in the Hilsa sex ratio. Prior to and during the prohibition, the average proportion of male Hilsa ranged from 3-16%, increasing to 35-

45% during the ban period (12 October- 2 November, 2023). Conversely, before and during the ban period, the average proportion of female Hilsa was 55-89%, rising to 85-95% during the ban period.
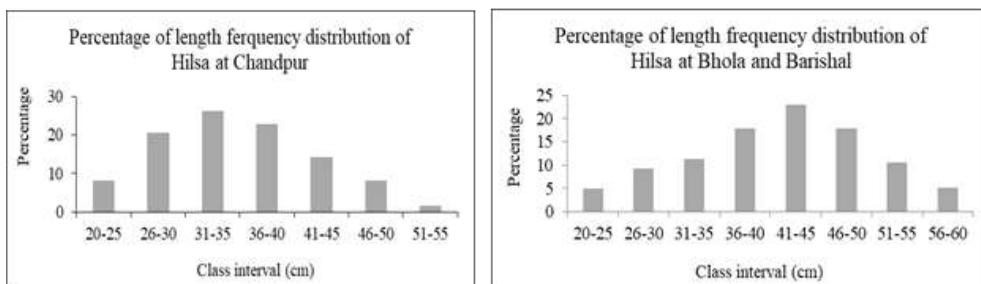

Fig. 3. Percentage of length frequency distribution of Hilsa at Chandpur and Bhola before, after and during the ban period (12 October- 2 November, 2023)
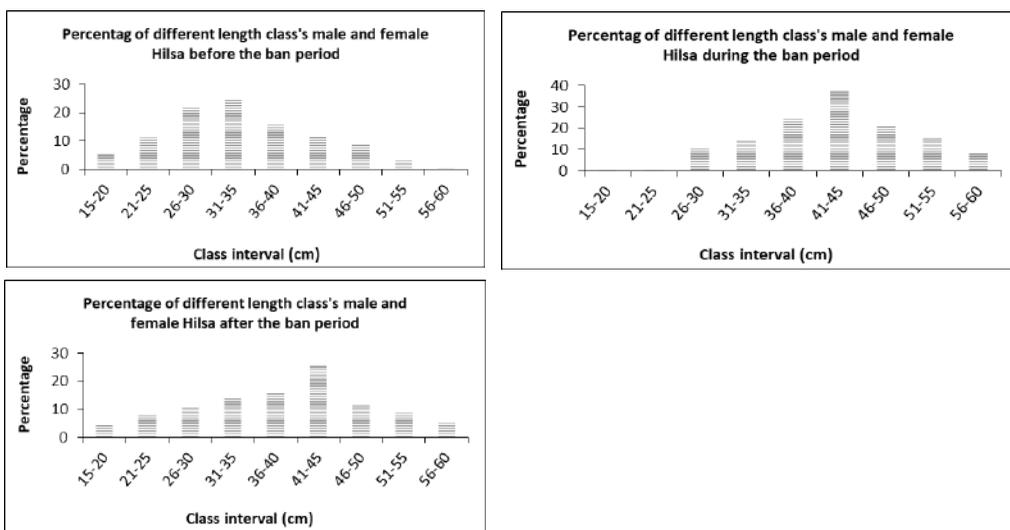

Fig. 4. Percentage of different length class's male and female Hilsa before, after and during the ban period (12 October-2 November, 2023)

These findings differ somewhat from those reported by Haldar (2004), who observed a male-female sex ratio of 1:2 in the main Hilsa spawning sites. In a separate study, Islam *et al.* (1987) found a male-female sex ratio of Hilsa to be 1:1.08 in the upper stretches of the Meghna river (Chandpur). Previous investigations conducted by BFRI revealed varying proportions, with Rahman *et al.* (2009) observing 35% male and 65% female, while Ahmad *et al.* (2010) found almost 4.2% male and 95.8% female among all

captured Hilsa in and around spawning grounds. During the monsoon, Quereshi (1968) noted a male-female sex ratio of 1:1, although females were dominant in October. According to Blaber *et al.* (2001), males are more abundant among smaller fish. The skewed sex ratio of *T. ilisha* suggests that males may have a shorter lifespan compared to females (Mahmud 2020). The majority of Hilsa collected through experimental fishing in this study were gravid females (80-90%) and larger than 40 cm, consistent with earlier findings. Consequently, it is evident that the fishing ban from October 12 to November 02 ensured effective breeding of approximately 80-90% of Hilsa.

Percent composition of spent and oozing Hilsa

In total, approximately 3000 Hilsa were examined in this study to assess breeding success. A significant number of both spent and oozing Hilsa were observed during the study period. In Ramgoti, 58.5% were oozing and 28% were spent; in Chandpur, the proportions were 16% and 41%, respectively; in Patuakhali, 17% were oozing and 64% were spent; and in Bhola, 36% were oozing and 74% were spent (Table 3). Combining all sample locations, the overall fractions of oozing and spent Hilsa were 31.7% and 52.4%, respectively, totaling approximately 84.1%. These results suggest that the fishing ban during the peak spawning season contributed to the successful breeding of a significant number of Hilsa. Similar positive impacts of fishing bans were reported by Ahmed *et al.* (2010), Rahman *et al,* (2011, 2012, and 2017) and Zaher *et al.* (2013) in their respective studies.

Table 3. Percent composition of spent and oozing Hilsa during the enclosure period

Status of Hilsa breeding	Sampling points					
	Cox's Bazar	Ramgoti	Chandpur	Potuakhali	Bhola	Average
Oozing	91 (31%)	506(58.5%)	86 (16%)	70 (17%)	302 (36%)	31.7%
Spent	162 (55%)	242 (28%)	221 (41%)	261 (64%)	621 (74%)	52.4%
Total Number	293	865	537	408	839	2942
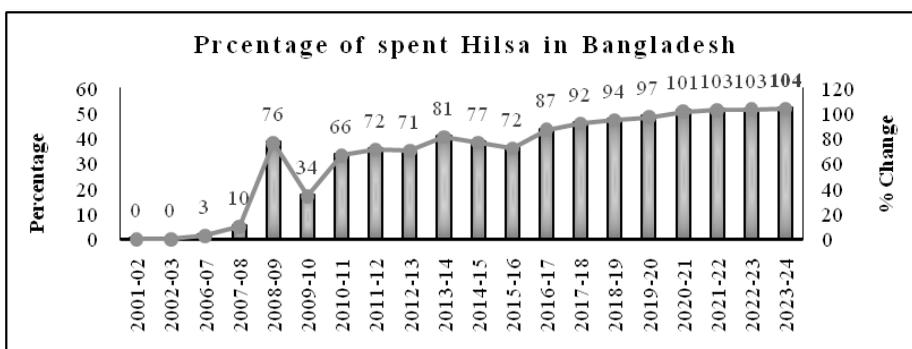
Fig. 5. Percentage and change of spent Hilsa in different years in Bangladesh.

In 2001-02, the percentage of spent Hilsa was >1%. Fishing bans were introduced during peak spawning seasons in 2006-07, resulting in the percentage of spent Hilsa increasing from 11% to 77%. However, it declined in the subsequent year (2008-09), possibly due to the severe impact of cyclone "Sidr" on coastal ecology and its dependent flora and fauna. Challenges in data collection also ascended due to coastal infrastructure loss during this period. From 2009-10 onwards, the percentage of spent Hilsa steadily increased until recent years (104.8% higher in 2023-24 than in 2001-02) due to the successful implementation of fishing bans during peak spawning seasons (Fig. 5). Rahman *et al.* (2009) found 1.51% oozing and 5% spent Hilsa during fishing ban periods in spawning grounds, which was 2.80-3.57 times higher than the studies conducted by the Global Environment Facility (GEF) and Bangladesh Fisheries Research Institute (BFRI) in 2002 and 2003. The occurrence of spent Hilsa ranged between 5-39% in 2007-09, estimated to be 76% higher than previous studies (Ahmed *et al.* 2010). Subsequent percentages of spent Hilsa were approximately 34% in 2010; 36% in 2011 and 2012; 41% in 2013; 39% in 2014; 37% in 2015; and 44% in 2016 (Rahman *et al.* 2017).

Production of fertilized eggs and jatka during the fishing enclosure period & spawning success

Due to fishing ban, approximately 8,11,711 kg of Hilsa eggs were produced in 2023-24 (Fig. 6). Assuming 50% of these eggs were fertilized and hatched, with a 10% survival rate, an estimated 4,05,855 crores of Hilsa fry and 40.58 crores of jatka were recruited into the Hilsa population.
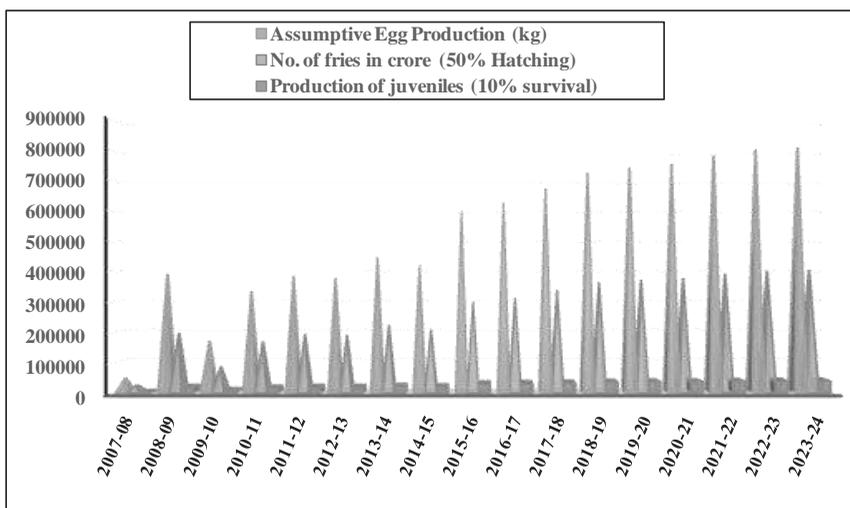
Fig. 6. Approximate number of eggs, fry and *jatka* production in the major spawning grounds.

These findings suggested that increasing the number of enclosure days (10 days from 2007-08 to 2010-11; 11 days from 2011-12 to 2013-14; 15 days from 2014-15 to 2015-16; and 22 days from 2016-17 to 2020-21) positively influenced egg and jatka production. In the current year (2023-24), egg production increased about 17 times, while fry and jatka production rose approximately 17.3 times compared to 2007-08, when fishing bans during peak spawning periods were initiated. Each year, the recruitment of a significant number of jatka into the Hilsa population as a result of fishing restrictions led to consistent and increased Hilsa production in Bangladesh (Figure 14). Studies by Haldar (2004), Ahmed *et al.,* (2010), Rahman *et al.,* (2011, 2012, 2017), Jaher *et al.,* (2013), and Mahmud *et al.,* (2020) have highlighted the significant positive impact of total fishing prohibition on jatka abundance and the overall Hilsa production in the country. Seasonal ban during the breeding or spawning periods directly reduces fishing mortality, thus achieving greater annual reproductive output (Murawski *et al.,* 2000; Arendse *et al.,* 2007).

CPUE of Hilsa fry and jatka

The use of fishing ban during Hilsa spawning seasons has shown notable benefits, particularly in the increase of catch per unit effort (CPUE) of jatka. This is evident in the significant rise in CPUE observed in Bangladesh, with the conservation efforts starting in 2003-04 and yielding tangible results by 2004-05, as reflected in the increased number of jatka per unit capture. In the current year (2023-24), the CPUE of jatka has demonstrated a substantial 42-fold increment compared to the base year (2004-05), and it's approximately 1.03 times higher than the previous year (2019-20) (Fig. 7). This increase underscores the success of Hilsa spawning due to fishing ban, leading to a greater availability of jatka in the country's waters.
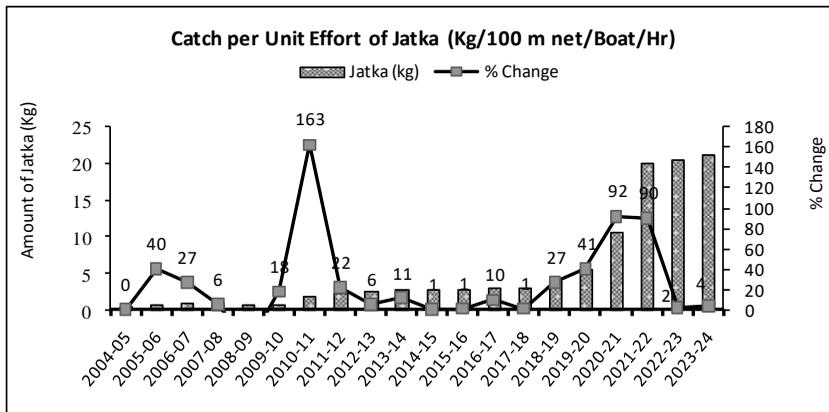
Fig. 7. CPUE of *jatka* in the selected sampling sites in different years.

Similar positive impacts of fishing ban on CPUE have been observed in various other regions worldwide. Studies such as those by Alam *et al.* (2019a, 2019b), Mahmud *et al.,* (2020), Oliver *et al.* (2015), Bagsit *et al.* (2021), Worm *et al.* (2006), *Roberts et al.* (2001), Kaunda-Arara & Rose (2004), Stobart *et al.* (2009) and Kamal *et al.* (2015) have highlighted the benefits of fishing ban on fishery yields, including increased CPUE. For instance, Oliver *et al.* (2015) noted significant increases in Octopus landings and CPUE following the reopening of ban in southwest Madagascar. Similarly, Bagsit *et al.* (2021) reported a significant rise in sardine catch at the conclusion of seasonal closures in the Visayan Sea, Philippines. Additionally, Worm *et al.* (2006) summarized studies demonstrating gains in CPUE associated with the dispersion or spillover of adult fishes from closed areas, as observed in the research by Roberts *et al.* (2001), Kaunda-Arara & Rose (2004) and Stobart *et al.* (2009). Moreover, Kamal *et al.* (2015) found significantly higher CPUE while investigating the effects of seasonal closures in a multispecies fishery. Overall, these findings underscore the effectiveness of fishing ban in enhancing fishery yields and promoting sustainable fishing practices by allowing fish populations to replenish during critical spawning seasons.

Total Hilsa production of the country

The implementation of management actions such as jatka conservation and fishing ban activation has significantly contributed to the remarkable increase in Bangladesh's Hilsa production. From 199,032 MT in 2002-03 to 571,000 MT by 2022-23, representing a 2.87-fold rise since 2002-03, this growth underscores the efficacy of these measures, particularly the enforcement of fishing ban. Research by the Bangladesh Fisheries Research Institute and Beets and Manuel (2007) accentuates the effectiveness of such measures in enhancing annual production and conserving fishery resources. Moreover, our analysis reveals a robust positive correlation (r = 0.879) between annual Hilsa catch and spent fish percentage (indicated spawning success), emphasizing the importance of holistic fisheries management approaches (Fig. 8).
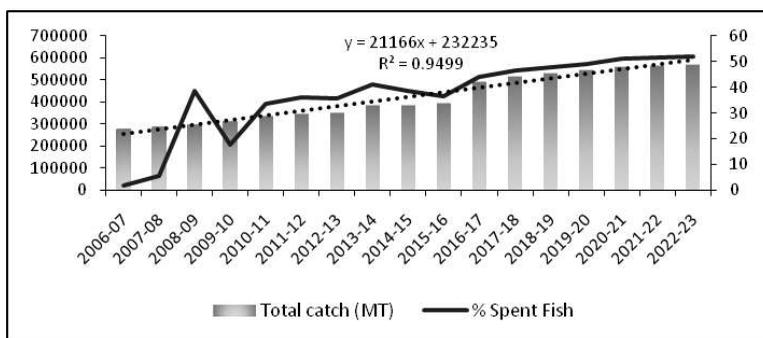

Fig. 8. Relation between total Hilsa production and spent fish percentage.

Availability of Hilsa and other fish's larvae (indicator of breeding success)

In the breeding grounds, a glass nylon fiber net was utilized to collect larvae during high and low tide conditions. During low tide, approximately 32% of Hilsa larvae were identified in the experimental net, while 68% comprised larvae of other fish species. Conversely, during high tide, Hilsa larvae accounted for 57%, with the remaining 43% being larvae of other fish species. Overall, 89% of Hilsa larvae were observed during both high and low tides (Fig. 9). The total number of larvae collected during high and low tides exhibited significant variation, with a higher number observed during high tide conditions (Fig. 10). The larvae of Shrimp, Chewa, Poa, Shillong, Kakila, Baim, Chanda, Kuchia, Bele, and other fish species were found alongside the larvae of Hilsa. After the breeding season, Hilsa larvae begin migrating to primary nursery grounds, where they undergo development into juveniles and subsequently jatka. Within a month, Hilsa larvae typically grow from 2 to 2.5 cm (Haldar 2004). The presence of Hilsa juveniles in various nursery grounds/sanctuary areas is indicative of successful Hilsa spawning. During BFRI's experimental fishing, a substantial number of Hilsa juveniles were discovered in nursery grounds, estimated to be aged between 30-45 days. Rahman *et al.,* (2017) reported a similar positive impact of fishing closure on the abundance of Hilsa and other fish larvae. In another study, Kincaid and Rose (2017) noted significant changes in biological community assemblages before–after and inside–outside the closure time and area. Kamal *et al.* (2015) investigated the effects of seasonal closures in a multi-specific fishery, highlighting positive biological effects on some target species depending on the timing of the ban period.
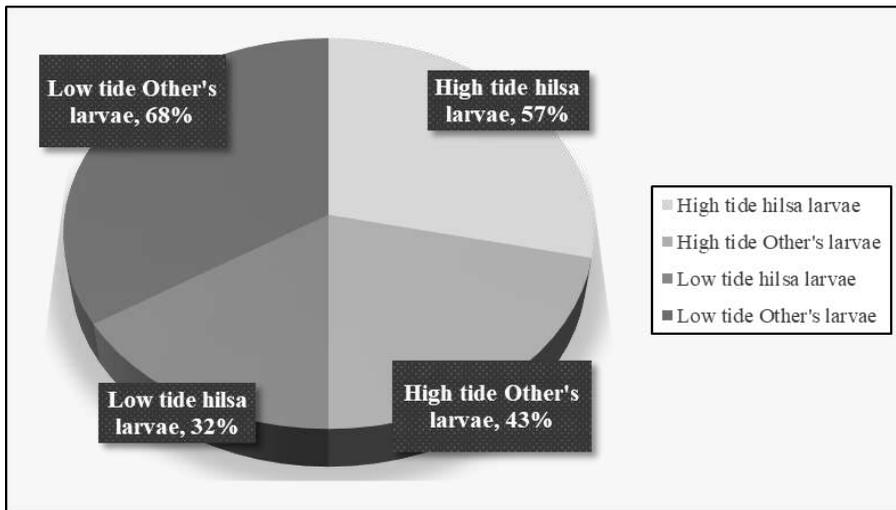
Fig. 9. Percentage of Hilsa and other fish larvae during high and low tide.
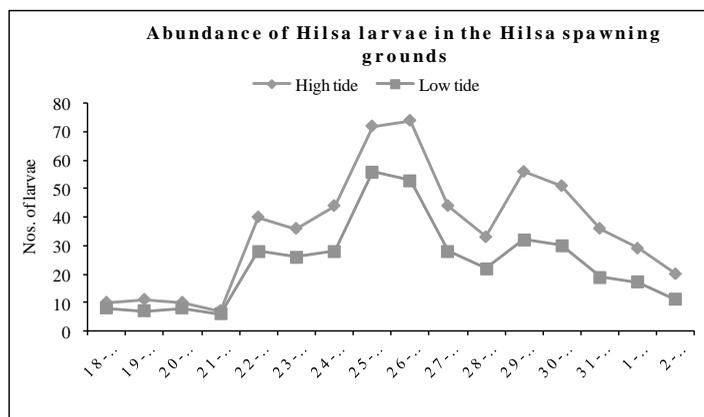
Fig. 10. Abundance of Hilsa larvae in the spawning grounds during fishing ban period (12 October- 2 November, 2023)

Conclusions and Recommendations

The enforcement of a 22-day fishing ban has emerged as a highly effective strategy, fostering successful Hilsa spawning and enhancing jatka production in major spawning grounds. However, it's important to note that the findings presented herein are approximations and may necessitate adjustments based on various factors such as fishing boat numbers, non-fishing participants, and sample sizes used for data collection. Additionally, numerous biotic and abiotic factors, including plankton composition, water quality parameters, and environmental conditions, could significantly impact Hilsa breeding success. Identifying the most influential factors would require further controlled experiments or extensive ecological assessments. Nonetheless, the observed increase in Hilsa production following management interventions suggests positive outcomes from these efforts. Based on the conclusion above, the following recommendations can be proposed:

- **Continuation of fishing bans:** It is advisable to continue implementing fishing bans to safeguard mature gravid Hilsa and protect jatka during critical breeding periods.
- **Implementation of fishing bans:** Fishing enclosures should be established in conjunction with other management interventions such as gear restrictions and area closures to further enhance Hilsa conservation efforts.

- **Effective management of spawning and nursery grounds:** Existing spawning grounds and nursery areas should be effectively protected and managed to ensure the sustained health and productivity of Hilsa populations.
- **Exploration of new breeding sites:** Further research is needed to identify potential new Hilsa nursery and breeding sites, which could contribute to the expansion of conservation efforts and the enhancement of Hilsa populations.
- **Enhanced support for research:** Increased logistical support for institutions like BFRI is essential to conduct comprehensive studies on the impact of fishing bans and other conservation measures on Hilsa populations.

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Risk assessment of organochlorine pesticides to human health and shrimp in southwest coastal region of Bangladesh

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Abstract

The goal of this experiment was to investigate the bioaccumulation of certain organochlorine pesticide residues in cultured and wild shrimp using an Electron Capture Detector (ECD) of Gas Chromatography. In this study, a total of eight sampling sites were chosen, including Bagerhat Sadar, Kochua, Chitolmari, Fakirhat, Mollahat, Mongla, Morelganj and Saronkhola Upazilas of Bagerhat district. Samples of shrimp and prawn were taken from these locations to assess the levels of different organochlorine pesticides, such as DDT, heptachlor, endrin and dieldrin. It was found that none of the pesticides exceed the maximum residual level (MRL) and was in very low concentration. The residue concentration in collected shrimp samples of heptachlor, endrin, dieldrin and DDT was 0.002~0.003ppm, 0.002~0.004 ppm, 0.002~0.005 ppm and 0.001 ppm residue respectively. However, among the available pesticides, percentage of heptachlor found higher (42%) and endrin seems to be less frequently available (9%). Monthly scenario of available pesticides indicated that in winter season (November-January), pesticides were more detectable than the other months. Risk assessment showed that shrimp samples from cultured and wild sources were safe for human consumption because all pesticides were minimal.

Keywords: Shrimp, Pesticide, Bioaccumulation, Gas Chromatography

Introduction

Bangladesh is one of the first countries to export frozen sea food to the United States, the European Union, Japan, Saudi Arabia, the United Arab Emirates, and Gulf states (Ali *et al.* 2012 and Khan *et al.* 2012). However, exporting countries frequently reject exported shrimp due to the presence of hazardous compounds. Due to poor hygiene practices and low-quality output, many seafood companies have closed their doors. After December 17, 1997, it was required to prepare all seafood according to the HACCP requirements (USFDA 1997). However, the implementation of HACCP in a processing facility involves more than just a narrative work. The HACCP system cannot be adopted until Good Manufacturing Practices (GMPs), Good Aquaculture Practices (GAPs), and sanitation criteria are met. Principles, procedures, and protocols known as "good

aquaculture practices" (GAP) are in place to support the growth of aquaculture in a way that doesn't harm the environment or its inhabitants, as well as to guarantee the product's high quality, safety, and longevity (Schwarz *et al.* 2010).

All persistent organic pollutants (POPs) have been banned in Bangladesh, including DDT and heptachlor, however some POP pesticides are still found (El-Mekkawi *et al*. 2009). The majority of farmers in the south-west region of Bangladesh, which includes the districts of Khulna, Bagerhat, and Shatkhira, cultivate shrimp and prawn with rice. To manage the pests in their rice fields, which finally accumulate in sediment and are transmitted to the bodies of shrimp and prawn via sediment, they must utilize a wide variety of pesticide classes. Organochlorine (OC) insecticides are among the most widely used agricultural pesticides for a very long time. They have been utilized widely in agriculture and in programs to control termites, mosquitoes, and tsetse flies (Guo *et al.* 2008). Numerous OC insecticides possess residues and metabolites with lengthy half-lives in the environment (ESDO 2005). Therefore, the purpose of this study was to determine the persistence of organochlorine insecticides in shrimp and prawns and the associated risks to human health.

Materials and Methods

Study area and sample collection
The study was carried out in 8 Upazillas of Bagerhat such as Kochua, Chitolmari, Fakirhat, Mollahat, Sarankhola, Mongla, Morelganj and Bagerhat Sadar upazilla during August 2021 to June 2022.

Shrimp/prawn samples were collected randomly from the wild and cultured areas from the sampling sites for assessment of pesticidal residues. During the course of the investigation, 500 g of shrimp and prawn samples were collected from both wild and cultured areas for the purpose of analyzing pesticidal residue. Following that, the samples were put through the GC-MS Machine's regular analysis process in order to check for the presence of DDT, dieldrin, endrin, and heptachlor. For the purpose of finding pesticide residues in wild and farmed shrimp, samples were collected at 30 days' interval.

Equipments: Fish mincer (Weisser No. 81 K), 500 mL and 100 mL round-bottomed flasks, Homogeniger IKAR T25 digital ULTRA-Turrax, a 50 mL and 10 mL volumetric flask, a nitrogen evaporator (N-EVAPTM111), a SPE cartridge (C18-REC 300 mg/3 mL), a magnetic starrier, and a gas chromatograph (GC-2010, Shimadzu).

Chemicals and reagents: Methanol, n-Hexane, Ethyl acetate/Acetonitrile, Primary Secondary Amine (all 99.99% pure, HPLC grade) and anhydrous sodium sulphate, anhydrous magnesium sulphate were acquired from Merk Company (Germany). Sigma Aldrich Chemicals provided the reference standards for DDT, dieldrin, heptachlor, and endrin (USA).

Procedure for extraction and cleanup for GC-MS/MS-based pesticide residue analysis:
The extraction was performed in accordance with the QUEschERS method, with the required modifications made for sample extraction, separation, and cleaning. In a Teflon tube, 10 grams of shrimp/prawn were taken. Then 20 mL of aceto-nitrile were added, and the mixture was agitated by hand for a full minute. The mixture was then agitated with a vortex for two minutes. After that 1.5g NaCl and 6g anhydrous $MgSO_4$ were added and mixed properly. Then, the mixture was shaken with a vortex for 30 seconds. The mixture was then filtered with 20g $NaSO_4$ and 10 mL Ethyl acetate anhydrous. The mixture was then centrifuged for 5 minutes at 5000 rpm. The supernatant was then collected in a flask with a round bottom, totaling 10mL. At a temperature of no greater than 400 degrees Celsius, the supernatant was evaporated using a rotary evaporator. A flask with a round bottom was filled with 5mL of n-Hexane. Then, 2 mL of n-hexane solution was added to a test tube. The n-hexane solution was diluted to a volume of 2 mL using 2 mL of H_2SO_4. It was then vortexed for one minute. After vortex, the mixture was centrifuged at 4000 rpm for three minutes. A 0.45 μm syringe filter was placed in a vial, and the supernatant was filtered through it into a tube. 2 mL sample was then taken and placed in a vial for GC-ECD or GC-FID analysis.

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Tissue Collection & Chopping
↓
Addition of Ethyl acetate or acetonitrile
↓
Filtration & Addition of sodium sulfate and Magnesium sulfate
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Pipetting of non-aqueous phase & drying through rotary evaporation
↓
Reconstitute & Filtration for GCMS/GC-ECD Injection

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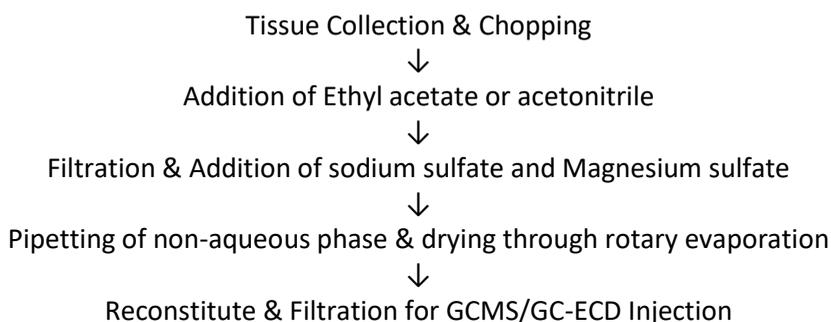
Fig. 1. Flow chart of sample extraction for Pesticides (Anastassiades *et al. 2*003)

Pesticide residues' impact and risk evaluation on human health
Data was evaluated separately for each shrimp/prawn species gathered. For each pollutant, a cumulative risk assessment was performed (pesticides). The risk evaluation

was based on consumption rates for important species at the local and national levels (DoF 2022). Two equations developed by USEPA (2000) were utilized to establish consumption recommendations for the analyzed fish species.

$$CR_{lim} = \frac{ARL \times BW}{CSF \times Cm} \qquad CRmm = \frac{CRlim \times Tap}{Ms}$$

Where, CR_{mm} is the most fish that can be eaten at one time (meals per year). CR_{lim} is the maximum amount of fish that can be eaten (in kilograms per day). Tap is the average number of days in a year (365.25), and MS is the meal (0.227kg of fish per meal).

NB: Cancer Slope Factor (CSF): 0.34 for DDT, 2.0 for other pesticides (Hardell *et al*. 2010), Bangladeshis eat an average of 63 g of fish every day (DoF 2022)

Results

The results of the study revealed that although pesticides were used in the shrimp and prawn farming industry in Bagerhat, the levels of residues detected in the samples were relatively low. The analysis identified trace amounts of DDT, dieldrin, endrin, and heptachlor in the samples collected from Morelganj, Sarankhola, Mongla, Kochua, Chitalmari, Fakirhat, Mollahat, and Bagerhat sadar upazillas. The accumulation of pesticide residue is stated in Table 1.

Table 1. Accumulation of pesticides residues in shrimp of different sampling sites of Bagerhat

Source	Targeted pesticides (ppm)	Study area (Upazillas in Bagerhat District)							
		Kachua	Chitolmari	Fakirhat	Mollahat	Mongla	Saronkhola	Morelganj	Bagerhat Sadar
Cultured Shrimp	Heptachlor	0.003 ±0.002	0.002 ±0.001	0.002 ±0.001	0.004 ±0.001	0.001 ±0.001	0.001 ±0.002	0.003 ±0.002	0.01 ±0.01
	Dieldrin	0.002 ±0.001	0.001 ±0.001	0.002 ±0.001	0.001 ±0.001	0.002 ±0.001	0.002 ±0.001	0.002 ±0.001	0.004 ±0.02
	Endrin	0.004 ±0.001	0.002 ±0.001	0.001 ±0.001	0.002 ±0.001	0.004 ±0.001	0.004 ±0.001	0.004 ±0.001	0.0085 ±0.005
	DDT	0.002 ±0.001	0.001 ±0.001	0.002 ±0.001	0.001 ±0.001	0.00	0.00	0.00	0.00
Wild Shrimp	Heptachlor	0.002 ±0.001	0.003 ±0.001	0.002 ±0.001	0.004 ±0.001	0.003 ±0.001	0.002 ±0.001	0.001± 0.001	0.002 ±0.001
	Dieldrin	0.005 ±0.002	0.002 ±0.002	0.003 ±0.002	0.002 ±0.002	0.002 ±0.002	0.004 ±0.002	0.002 ±0.002	0.005 ±0.002
	Endrin	0.0002 ±0.001	0.002 ±0.001	0.001 ±0.001	0.0002 ±0.002	0.0002 ±0.001	0.0002 ±0.001	0.0002 ±0.001	0.0002 ±0.001
	DDT	0.003 ±0.001	0.002 ±0.001	0.001 ±0.001	0.0023 ±0.001	0.00	0.00	0.00	0.00

Maximum residual level is not exceeded by any of the samples. Among our four targeted pesticides, Heptachlor was frequently found.
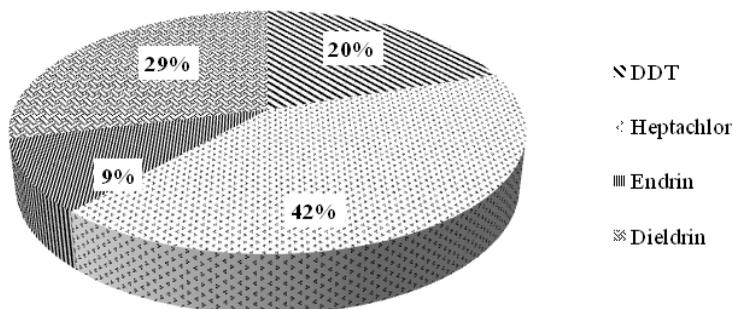

Fig. 2. Ratios of available pesticides (below Maximum residual level) in our study area.

During the colder months of the year specially from November to January, pesticide residues were detected more frequently (but did not exceed the allowed level), whereas they were found less frequently during the warmer months of the year.
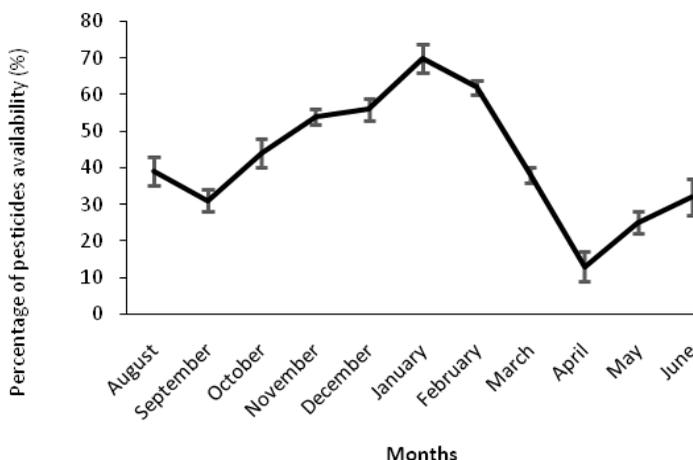

Fig. 3. Monthly Scenario of available targeted pesticides in study area.

Among our study areas, the samples from Kachua Upazila were found to contain pesticides more frequently, while fewer instances of pesticides were found in samples from Sarankhola Upazila. However, none of the samples exceeded the alarming concentration of pesticides.
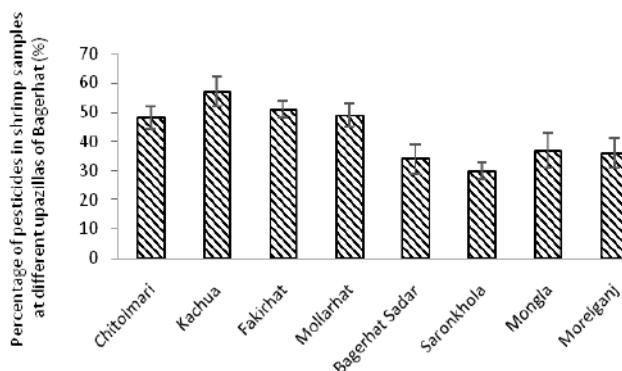
Fig. 4. Scenario of available pesticides in different sampling sites.

Human health risk evaluation of these accumulated pesticide residues

The results of a risk assessment of pesticide accumulation in samples of farmed and wild shrimp are shown below (Tables 2 and 3).

Table 2. Accumulation of pesticide residues in cultured shrimp and the risk-based rate of human intake

Sampling Sites	Pesticides	ARL	BW	CSF	Pesticides concentration C_m (ppm)	CR_{lim} (kg/day)	CR_{mm} (Kg/year)
Kachua		0.00001	55	2	0.003	0.09	521.7
Chitolmari		0.00001	55	2	0.002	0.14	811.6
Fakirhat		0.00001	55	2	0.002	0.14	811.6
Mollahat	Heptachlor	0.00001	55	2	0.004	0.07	405.8
Mongla		0.00001	55	2	0.001	0.275	1594.3
Saronkhola		0.00001	55	2	0.001	0.275	1594.3
Morelganj		0.00001	55	2	0.003	0.09	521.7
Bagerhat Sadar		0.00001	55	2	0.01	0.02	159.43
Kachua		0.00001	55	2	0.0021	0.13	759.21
Chitolmari		0.00001	55	2	0.0011	0.25	1449.40
Fakirhat	Dieldrin	0.00001	55	2	0.0023	0.12	693.19
Mollahat		0.00001	55	2	0.0011	0.25	1449.40
Mongla		0.00001	55	2	0.0024	0.11	664.31
Saronkhola		0.00001	55	2	0.002	0.14	797.17
Morelganj		0.00001	55	2	0.002	0.14	797.17
Bagerhat Sadar		0.00001	55	2	0.004	0.07	398.59
Kachua		0.00001	55	2	0.004	0.07	398.59
Chitolmari		0.00001	55	2	0.002	0.14	797.17
Fakirhat	Eldrin	0.00001	55	2	0.001	0.28	1594.35
Mollahat		0.00001	55	2	0.002	0.14	797.17
Mongla		0.00001	55	2	0.004	0.07	398.59

Saronkhola		0.00001	55	2	0.004	0.07	398.59
Morelganj		0.00001	55	2	0.004	0.07	398.59
Bagerhat Sadar		0.00001	55	2	0.0085	0.03	187.57
Kachua		0.00001	55	0.34	0.002	0.81	4689.25
Chitolmari		0.00001	55	0.34	0.001	1.62	9378.50
Fakirhat		0.00001	55	0.34	0.002	0.81	4689.25
Mollahat		0.00001	55	0.34	0.001	1.62	9378.50
Mongla	DDT	0.00001	55	0.34	0	-	-
Saronkhola		0.00001	55	0.34	0	-	-
Morelganj		0.00001	55	0.34	0	-	-
Bagerhat Sadar		0.00001	55	0.34	0	-	-

Table 3: Accumulation of pesticide residues in wild shrimp and the risk-based rate of human intake

Sampling Sites	Pesticides	ARL	BW	CSF	Pesticides concentration C_n (ppm)	CR_{lim} (kg/day)	CR_{mm} (Kg/year)
Kachua		0.00001	55	2	0.002	0.14	797.17
Chitolmari		0.00001	55	2	0.003	0.09	531.45
Fakirhat		0.00001	55	2	0.002	0.14	797.17
Mollahat		0.00001	55	2	0.004	0.07	398.59
Mongla	Heptachlor	0.00001	55	2	0.003	0.09	531.45
Saronkhola		0.00001	55	2	0.002	0.14	797.17
Morelganj		0.00001	55	2	0.001	0.28	1594.35
Bagerhat Sadar		0.00001	55	2	0.002	0.14	797.17
Kachua		0.00001	55	2	0.002	0.14	797.17
Chitolmari	Dieldrin	0.00001	55	2	0.001	0.28	1594.35
Fakirhat		0.00001	55	2	0.002	0.14	797.17
Mollahat		0.00001	55	2	0.001	0.28	1594.35
Mongla		0.00001	55	2	0.002	0.14	797.17
Saronkhola		0.00001	55	2	0.002	0.14	797.17
Morelganj		0.00001	55	2	0.002	0.14	797.17
Bagerhat Sadar		0.00001	55	2	0.004	0.07	398.59
Kachua		0.00001	55	2	0.0002	1.38	7971.73
Chitolmari		0.00001	55	2	0.002	0.14	797.17
Fakirhat		0.00001	55	2	0.001	0.28	1594.35
Mollahat		0.00001	55	2	0.0002	1.38	7971.73
Mongla	Eldrin	0.00001	55	2	0.0002	1.38	7971.73
Saronkhola		0.00001	55	2	0.0002	1.38	7971.73
Morelganj		0.00001	55	2	0.0002	1.38	7971.73
Bagerhat Sadar		0.00001	55	2	0.0002	1.38	7971.73
Kachua		0.00001	55	0.34	0.003	0.54	3126.17
Chitolmari		0.00001	55	0.34	0.002	0.81	4689.25
Fakirhat		0.00001	55	0.34	0.001	1.62	9378.50
Mollahat		0.00001	55	0.34	0.0023	0.70	4077.61
Mongla	DDT	0.00001	55	0.34	0	-	-
Saronkhola		0.00001	55	0.34	0	-	-
Morelganj		0.00001	55	0.34	0	-	-
Bagerhat Sadar		0.00001	55	0.34	0	-	-

Discussion

Among the pesticide residue data obtained, none of the samples revealed alarming concentration levels in comparison to the European Union's permissible limit (EU). The permitted limit for heptachlor and endrin in shrimp/prawn is 0.01 ppm, according to the EU. Dieldrin has an acceptable level of 0.02 ppm in shrimp/prawn while DDT has an acceptable limit of 0.05 ppm in shrimp/prawn (Islam *et al.* 2015). In this study, it was found that all the pesticides concentration was very low in the shrimp samples. Although the concentration of pesticides found very much lower, the percentage of available heptachlor were higher (41.7%) than other three targeted pesticides in shrimp samples and the lower percentage from the samples were found in endrin (9.6%). However, lower amounts of pesticides were found because over 80% of the population in the southwest part of Bangladesh relies on shrimp/prawn culture with vegetables or rice for subsistence (Islam *et al.* 2015). It was observed in Bagerhat Sadar upazilla that if anyone consume shrimp up to 159.43 kg/year and accumulated with 0.01 ppm heptachlor residue then after one year anyone may fall into serious health risk like cancer. But this is quite impossible to consume that amount of shrimp in a year which indicates that the pesticides accumulation in shrimp is very much low in concentrations and not alarming for human consumption. Like heptachlor, the concentration of other pesticides in cultured and wild shrimp of other 7 upazillas of Bagerhat were found in very low concentration. Due to the longevity in the environment, most organochlorine pesticides (OCPs) were outlawed in the 1970s (Annonymous 1979 and Annonymous 1989). Pesticides, notably the widely used diphenyl trichloride (DDT), were prohibited in Bangladesh in 1995 after decades of abuse (Matin *et al.* 1998). OCPs have a lengthy half-life and can therefore be found in low concentrations in fish and shrimp from a wide range of water sources (Zhang *et al.* 2014, Prodhan *et al.* 2010, Prodhan *et al.* 2009, Kaur *et al*. 2008, Antunes and Gil 2004, Osuna-Flores and Riva 2001, Chan *et al.* 1999, Berg *et al*. 1999, Sapozhnikova *et al*. 2004).

This study also analyzed the availability of pesticides over a 12-month period which indicated that the months of November through January had the highest prevalence. Watterson (1991), Robertson *et al.* (2002), John *et al.* (2001), and Tsiplakou *et al.* (2001) found similar things. They reported that organochlorine pesticide residues were found to be more prevalent in winter samples compared to summer samples. Among our sampling sites, the high percentage of available pesticides in shrimp sample was found in Kachua upazila. This was happened due to the integrated vegetables farming and application of pesticides in that vegetable fields (Islam *et al.* 2017). Although some pesticides were banned, low concentration of that pesticides were found due to their longer persistence in the environment (Jayaraj *et al.* 2017)

This study aims to provide information on the bioaccumulation of organochlorine pesticides in shrimp samples collected from the designated sampling locations. According to the statistics, not a single sample, whether it came from natural or farmed sources, exceeded the level considered to be problematic. In point of fact, the number of pesticides present at each location where samples were taken was relatively negligible. These pesticides degrade slowly and remain in the environment for an extended period of time after they have been sprayed. It is necessary to conduct periodic checks of the pesticide levels in each intercompartment and to promote public awareness in order to combat the substantial problem of pollution generated by these organochlorine pesticides that accumulate in the food chain. In order to do so, it is necessary to raise awareness among the general public.

Acknowledgement

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Cage culture of Seabass (*Lates calcarifer*) in Cox's Bazar coast of the Bay of Bengal: A pilot study

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Abstract

The coastal fishery resources of Bangladesh are being depleted rapidly, although brackish water resources are nutrient-rich and biologically diverse. Implementing mariculture in coastal environments could be a timely approach to utilizing these productive resources. In this regard, a pilot study on the cage culture of seabass (*Lates calcarifer*) in Cox's Bazar coast of the Bay of Bengal was conducted to study the feasibility. Initially, 170 ± 6.53 g fish were stocked with a density of 10 Ind/m^3. Chopped sardine fish (*Sardinella fimbriata)* was used as feed at the rate of 5% body weight. The growth parameters i.e. final weight (936 ± 8.65 g), survival rate (75%), average daily growth rate (4.75 g/day), potential weight gain (451 g) and feed conversion ratio (12.79 ± 0.55) have shown that the culture system is technically feasible. In addition, the water quality parameters were found suitable for cage culture of seabass throughout the experimental period. Proper optimization of this technology will be a proficient step in utilizing vast coastal water for mariculture practices.

Keywords: Cage farming, *Lates calcarifer*, Cox's Bazar coast

Introduction

The marine resources of Bangladesh are rich in biodiversity and support the livelihood of a large number of coastal people (Islam *et al.* 2022). The production of marine fish is declining, indicating that the marine fish stocks are under severe exploitation. So, it is necessary to develop new alternatives for fish production that don't affect wild stocks. In this regard, mariculture could be a great way to enhance production without affecting wild stocks.

Initiating cage culture or pen culture activities in coastal water with suitable species would enhance fish production in addition to some extra economic benefits (Aswathy and Imelda, 2019). Several studies have shown that cage aquaculture in coastal water is suitable for a variety of species considering its water quality parameters for fish culture (Krishna *et al.,* 2014). In addition, it also secures the livelihood of coastal fishers (Manju

Lekshmi, 2015). Furthermore, proper optimization of these systems maximizes coastal habitat fish production by utilizing a variety of natural feed (Vikas *et al.,* 2010). Despite all these potentials, some challenges have to be faced in the case of coastal cage culture here in Bangladesh. These are a lack of suitable seed and feed, a lack of financial support, and the impact of climate change.

Over the last 30 years, marine finfish culture in cages has been dramatically increasing. Seabass (*Lates calcarifer*) is a potential candidate for coastal cage culture. Seabass is mostly cultured in ponds adjacent to coastal areas or in cages located in estuaries or coastal areas. Though Bangladesh has a huge coastal area, there is not any coastal r cage culture system reported yet. Presently, the most pressing issue for Bangladesh in coastal cage culture is the 'selection of suitable culture area'. That is why; this pilot-scale coastal cage culture experiment was undertaken by Bangladesh Fisheries Research Institute (BFRI) at Coxˈs Bazar coast of the Bay of Bengal. The findings of the study will provide a general understanding of the coastal cage culture system.

Materials and Methods

Experimental site selection
A survey was conducted initially to select a suitable place for cage installation and farming. A suitable site at Moheskhali channel, Khurushkhul, in Coxˈs Bazar was selected for cage installation (Fig. 1). The selected site was also suitable for cage culture of seabass considering its physico-chemical profile according to Jayanthi and Kailasam (2019). The water quality, environmental and physical parameters are summarized in Table 1.

Table 1. Water quality, environmental and physical parameters of the selected site

Parameters	Values
Salinity	20-35 ppt
pH	7-8.5
Water temperature	20-32 °C
Dissolved oxygen	5-8 ppm
Transparency	25 cm
Water current	25-40 cm/s
Water depth	4-5 m
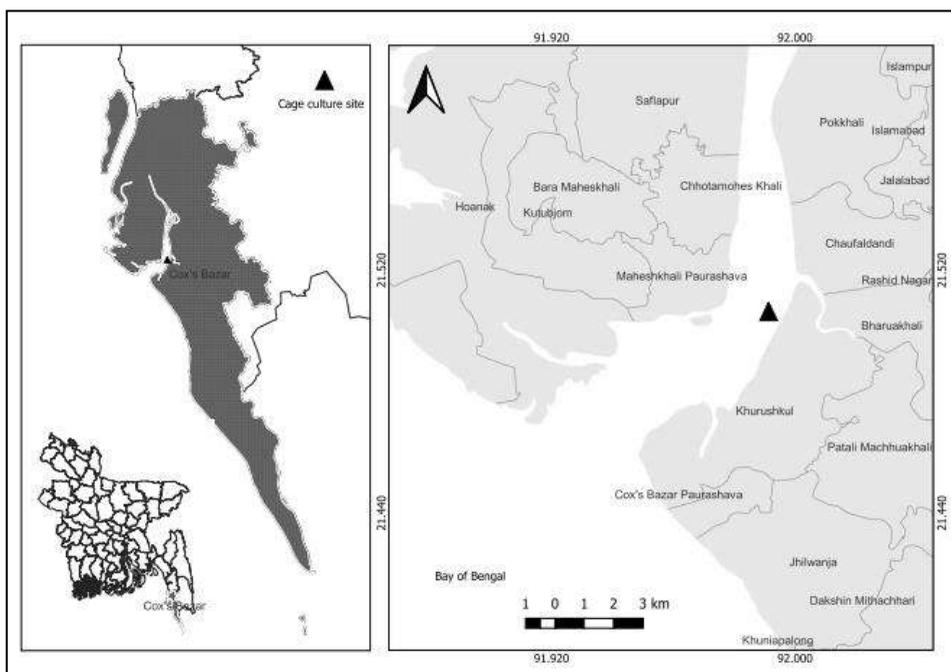
Fig. 1. The cage culture site (21° 30' 16.68"N and 91° 59' 39.39" E)

Cage description

The experimental circular cage frames were constructed with an outer and inner base floating collar, as well as a central support collar. The collars were built of PE100 PN 10 IS 4984 high-density polyethylene (HDPE) with a PE100 PN 10 standard. Concealed Plastic drums of 990 mm in length were used between the outer collars for floatation. Each cage of 48 m^3 was enclosed with a nylon net of 40 mm mesh sized for the rearing of fishes. A nylon net of 20 mm mesh size was used upon the enclosure net as the cover net. A net cover was used to protect the fish stock from predators. Nylon rope tied 4 moorings (50 kg each) was used as anchors to fix a cage (Fig. 2).

Stocking and rearing

In Bangladesh, no artificial breeding technology for seabass has yet been developed. That's why juvenile seabass was stocked from different local areas of Cox's Bazar district (Sonadia, Hashimchor, Choufoldondi, and Moheshkhali), where the farmer fills their tide-fed pond with tidal water during high tide via a sluice gate and culture extensively without providing any feed or food. The stocked fish ranged in size from 170 ± 6.53 grams, with a stocking density of 10 individuals/m^3. Chopped Sardines (*Sardinella*

fimbriata) were fed to the fish at a rate of 5% of their body weight. To allow the free flow of water, the nets were cleaned at regular intervals.

Fig. 2. Cage culture unit at culture site

Water quality

The water quality parameters like salinity, temperature, dissolved oxygen, pH, and ammonia were measured twice a month. The salinity, temperature, and dissolved oxygen were measured on the spot using a Hanna HI-9829 multiparameter. The water sample was collected and transported to the laboratory for ammonia test. The ammonia test was carried out using a Hanna multiparameter bench photometer (HI 83099).

Growth performance indices

The viability of the study was evaluated in terms of the growth performance of the stocked fish. The growth dynamics were calculated in terms of survival rate, average daily growth rate (ADGR), potential weight gain (PWG), specific growth rate (SGR), and feed conversion ratio (FCR). The following equations were used to calculate these growth performance indices;

1. Survival rate (%) = $\dfrac{Total\ number\ of\ fish\ harvested}{Total\ number\ of\ fish\ stocked} \times 100$

2. ADGR (g/d) = $\dfrac{Average\ final\ weight - Average\ initial\ weight}{Culture\ duration\ (days)}$

3. PWG (%) = $\dfrac{Average\ final\ weight - Average\ initial\ weight}{Average\ initial\ weight} \times 100$

4. SGR (%) = $\dfrac{ln\ final\ weight - ln\ initial\ weight}{Culure\ days} \times 100$

5. FCR = $\dfrac{Total\ feed\ fed}{Total\ weight\ gain}$

Statistical analysis

Descriptive statistics (mean, standard deviation) of the collected data were analyzed using Microsoft Excel (2016) for interpretation of the results.

Results and Discussion

Water quality

For a successful cage culture, optimal water quality is essential. The water quality parameters (salinity, temperature, dissolved oxygen, pH, and ammonia) observed during the study period are summarized in Table 2.

Table 2. Water quality parameters at the culture site during the experimental period

Months	Salinity (ppt)	Temperature (°C)	Dissolve Oxygen (ppm)	pH	Ammonia (ppm)
Jan, 21	32.55±0.30	21.50±0.15	5.65±0.29	7.2±0.10	0.02±0.002
Feb, 21	31.30±0.30	22.55±0.25	5.80±0.48	7.35±0.20	0.02±0.003
Mar, 21	31.65±0.30	24.20±0.10	5.85±0.14	7.15±0.20	0.015±0.003
Apr, 21	31.13±0.03	28.50±0.07	5.53±0.23	7.27±0.17	0.023±0.004
May, 21	28.57±0.27	28.77±0.17	5.83±0.13	7.43±0.13	0.013±0.003

*Values are mean ± Standard Error, where n = 6

During the experimental period, the salinity ranged between 28.8-32.6 ppt. As seabass is a euryhaline species, a wide salinity range is tolerable and suitable for seabass culture (McKenzie *et al.* 2001). The temperature ranged between 21.6-29.1 °C at the culture site. Tucker *et al.* (2002) reported seabass as a highly thermal tolerant species where expected growth and food conversion ratio are seen between 25-36 °C (Katersky and Carter 2005). The dissolved oxygen of the culture site ranged between 5.6-5.8 ppm and pH ranged between 7.2-7.4. All the observed water quality parameters of the culture site were within the recommended range according to Schipp *et al.* (2007) and Jayanthi and Kailasam (2019). The environmental conditions and physiological behaviour of the experimental fish pointed to a favourable environment for seabass coastal cage culture.

Growth performance indices

Growth performance indices of the stocked seabass in cages are shown in Table 3. After a 150-day rearing period, the mean survival rate was found 95% with 4.75 g average daily growth per day. Aswathy and Imelda (2018) reported 1080 kg seabass production from coastal cage culture with a stocking density of 32 ind/m³. In this experiment, comparatively low stocking density was maintained compared to other open sea cage cultures. That was because of the shallow water in the coastal area. The FCR value of the study was 1.8.

Table 3. Growth performance indices of the stocked seabass in cages

No.	Measures	Unit	Values
1	Initial stocking size	g	170 ± 6.53
2	Stocking density	Ind/m³	10
3	Survival rate	%	75%
4	Average daily growth rate (ADGR)	g/day	4.75
5	Potential weight gain (PWG)	%	451
6	Specific growth rate (SGR)	%	1.14 ± 0.11
7	Feed conversion ratio (FCR)	-	12.79± 0.55

*Values are mean ± Standard Error

The production during the final harvest was around 8 kg/m³. The average final weight at the time of harvest was reported around 936 g which varied from 800 g to 1000 g. Liyanage and Pushpalatha (2018) reported 22-26 kg/m³ seabass production from cage culture in the coastal lagoons. Around 75% survival rate was recorded after harvesting. This was found better considering the previous findings of 65% by Kailasam *et al.* (2001) and 68.8% by Philipose *et al.* (2013).

The findings of the study show that the coastal cage culture of seabass (*L. calcarifer*) has significant advantages in terms of fish production and utilization of coastal water. It directly can minimize the excess pressure on wild stocks. Besides, it could be a great alternative income source for the coastal fishers. Kumaran *et al.* (2019) reported the incorporation of a new aquaculture-based culture approach in coastal areas increased the ability to ensure livelihood security. Overall, coastal brackishwater cage culture technology showed great viability for implication as an aquaculture industry in the Cox's Bazar coast.

The results of the study indicate that seabass farming in coastal cage culture is technically possible. Further research regarding the cage culture of seabass and other finfish species should be conducted on a comparatively larger scale to firmly conclude on this matter.

Acknowledgment

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Current scenario of shrimp farming practices in the Southwestern region of Bangladesh: Challenges and opportunities

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Abstract

A study was conducted between July and December 2022 with a detailed face-to-face questionnaire survey to describe the coastal shrimp farming practices and management among different category of farms in southwestern Bangladesh. Three districts (Khulna, Bagerhat, and Satkhira) comprising around 180 shrimp farmers were chosen, and data were gathered using direct interview approaches. The production systems include multi-stock and multi-harvest throughout the year. Inputs provided into the ghers were mainly limited to inorganic fertilizer (Urea, TSP, DAP etc.) and some organic supplements. Average water depth was less than 3 feet with the water exchange of once in a month, but 6.67% of farmers in Satkhira region exchanged water twice in a month. Most of the farmers in Bagerhat maintained an average stocking density above 150 to 200 PL/decimal for *Penaeus monodon*. About 84.17% of shrimp farmers preferred a mixed culture of shrimp, prawn, and finfish in the ghers to increase biomass and to avert the risk of diseases in *P. monodon* monoculture. The study revealed that, In the Khulna and Satkhira regions, formulated feed was used more frequently than commercial feeds, whereas commercial feed was used in Bagerhat. Poor disease control strategy caused a 60 to 70% survival rate in maximum farms of Bagerhat and Khulna districts. The study suggests that proper management and planning following good aquaculture practices (GAPs) would ensure sustainable growth and benefit of shrimp farming which would bring immense socioeconomic and environmental impacts.
Keywords: Shrimp culture, *Penaeus monodon*, Southwest Bangladesh, GAP

Introduction

Bangladesh is known as the most favorable field for tiger shrimp (*Penaeus monodon*) farming all over the world. Due to advantageous environmental resources and agro-climatic circumstances, especially the accessibility of wild post-larvae, sub-tropical monsoonal climate, low-lying agricultural land, saline water availability, and a vast area of shallow water provided ideal conditions for shrimp production and shrimp farming is a common activity in coastal Bangladesh (Ahmed *et al*. 2008a, Ahmed 2013a, Ahmed and Diana *et al.* 2015). A subtropical environment with plenty of brackishwater offers a special possibility for shrimp farming. An estimated 262,980 acres of land in Bangladesh

is used for shrimp cultivation. Bangladesh's southwest is home to around 75% of the country's shrimp farms, which produced 63,171 tons of shrimp annually from an area of 185,308 acres in 2018–2019 (DoF 2023). In the period from 2021 to 2022, Bangladesh exported 74,043 tons of fish and fish products worth BDT 5196.76 crore, of which BDT 3636.59 crore (69.98%) came from the export of shrimp (DoF 2022). It makes a substantial economic contribution to the country as the second-largest export item after ready-made clothing.

The country is known as one of the most suitable places in the world for tiger shrimp (*Penaeus monodon*) aquaculture. This cultivation of shrimp has a high potential due to the excellent biophysical resources as well as agroclimatic conditions the country has to offer. The subtropical environment with vast amounts of brackishwater of Bangladesh offers special possibilities for shrimp farming. The easy access to wild post-larvae enhances this suitability. It is estimated that in 2020, 260,000 hectares of land is used for cultivation, of which 75% is located in the southwest and the remaining 25 percent found in the southeast (Ahmed 2015). Many shrimp/prawn farms have been established throughout Bangladesh, although the greater Khulna regions, which account for 74.5% of all shrimp/prawn farms in Bangladesh, produce the most (78%) (Mamun *et al*. 2020). The Satkhira district, where many farmers have transformed their rice fields into prosperous shrimp and prawn farms, has seen the most remarkable growth in shrimp farming (Ahmed *et al*. 2008b).

It is frequently asserted that Bangladeshi shrimp farming was unplanned and resulted in environmental degradation, such as decreased water and soil quality, decreased farm productivity due to decreased land and soil fertility, decreased livestock production due to decreased grazing land, risked human health, and destroyed mangrove forests. (Haque *et al*. 2023). However, several studies on shrimp farming in Bangladesh have been performed including technical efficiency (Begum *et al.* 2015), economic analysis (Alam *et al.* 2007), social and economic status (Alauddin and Hamid 1999), shrimp farmers' livelihood analysis (Mamun *et al.* 2019), changing in shrimp culture methods with an emphasis on disease (Parvez *et al*. 2006), etc. Despite this, we lack sufficient knowledge about the shrimp farmers' practices and the main issues they encounter. The purpose of this study was to determine the current status of shrimp farming in Southwestern region of Bangladesh as well as to make some recommendations for expanding shrimp output.

Materials and Methods

A well-designed questionnaire was prepared for the collection of relevant information following the objectives. Data were collected throughface-to-face questionnaire interview considering around 180 shrimp farmers from the selected upazilas of Bagerhat, Khulna, and Satkhira districts. Respondents were selected through random questionnaire surveys like Large Group Discussions (LGD), and Cross-Check interviews with Key Information (KI). Besides the primary information study, secondary data were collected from the Department of Fisheries (DOF) through Focus Group Discussion (FGD), Export Promotion Bureau (EPB), relevant journals, thesis, reports, and official records. The information obtained from the survey was accumulated, grouped, and interpreted according to the objectives as well as parameters. Some data contained numeric and narrative facts. The collected data were then coded, summarized, processed, and tabulated by using statistical tools like averages and percentages and analyzed by IBM SPSS Statistics 25 software.

Results

In Bagerhat maximum farmer had experience above 10 years but 50% of farmers had 1 to 5 years' experience in Khulna. On the other hand, most farmers had experience above 5 years to 10 years in Satkhira (Fig. 1).

Fig. 1. Culture experience of farmers of the study area.

Pre-stocking activities

Most of the farms had no canal in Bagerhat and Khulna but canal availability was better in Satkhira region (Fig. 2). In Bagerhat, the Maximum gher size was above 100 to 150 decimals and the maximum gher size was above 50 to 100 decimals in Khulna but in Satkhira maximum gher size was less than 50 decimals according to the field survey (Fig. 2). Most of the gher's average water depth in the surveyed area was less than or equal to 3 feet (Fig. 3). The river water was used as a water source in all the gher of Khulna and Bagerhat region but in Satkhira most of the gher used river water as the water source, groundwater and both.
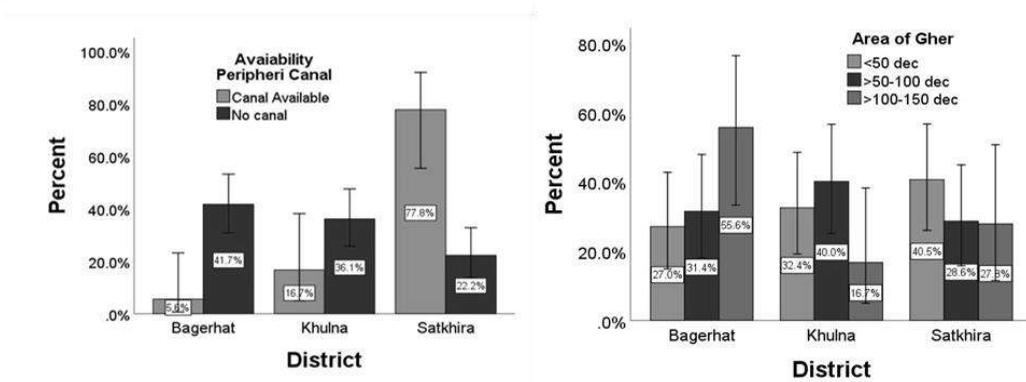
Fig. 2. Peripheri canal availability and *gher* area.
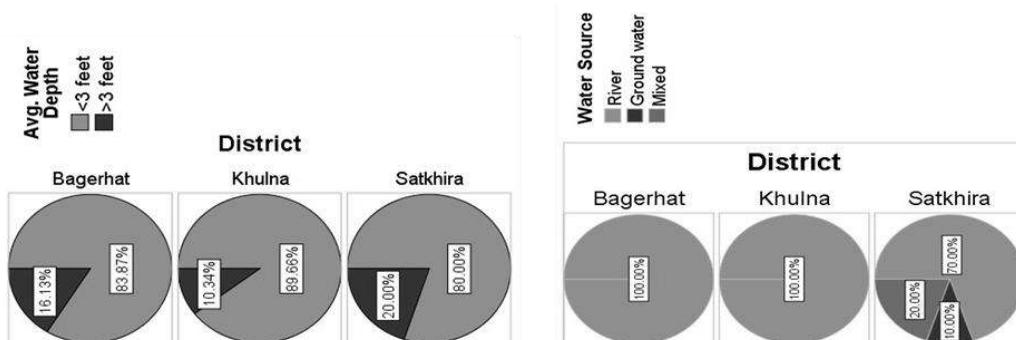
Fig. 3. Average water depth and source of water.

Water exchange of all of the gher had been1time/month but 6.67% of farmers in Satkhira region exchange water in gher 2 times/month. Most of the ghers in Bagerhat and Khulna region followed 5 steps of gher preparation method but in Satkhira region it is 50% (Fig. 4). In Bagerhat all the gher used inorganic fertilizer (Urea, TSP, DAP etc.) and in Khulna, all ghers used organic fertilizer but in Satkhira, equal proportion of gher used organic and inorganic (Fig. 4).
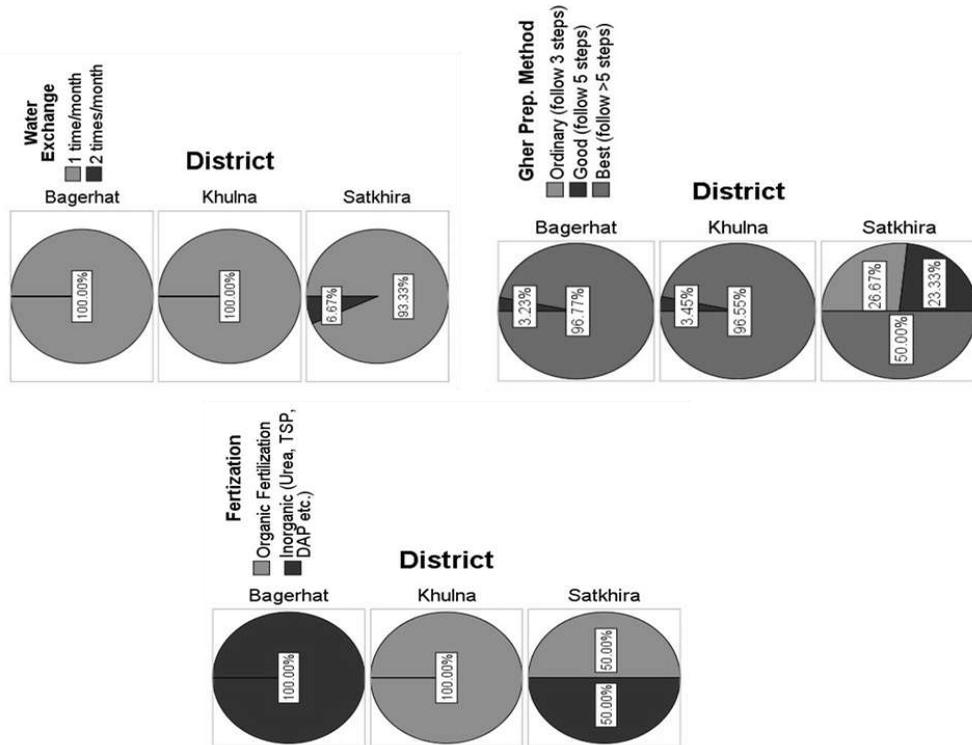
Fig. 4. Overview of water exchange, gher preparation method and fertilization uses.

Stocking management

From the study, it was found that 100% ghers of Bagerhat had been concmitantly cultured giant freshwater prawn, tiger shrimp, and white fishes but in Khulna and Satkhira regions monoculture of shrimp was found frequently (Fig. 5). In Bagerhat most of the ghers used hatchery produced SPF (Specific Pathogen Free) PL during stocking.
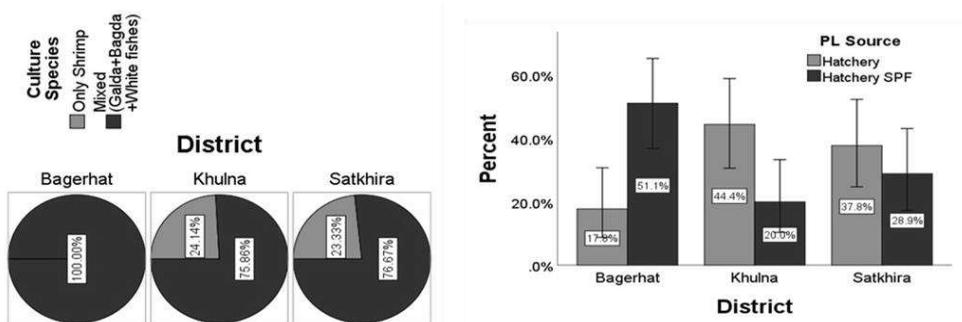
Fig. 5. Overview of culture species and PL source.

Most of the farmers in Bagerhat stocked above 150 to 200 PL/ decimal and above 100 to 150 PL/decimal in Khulna but in Satkhira stocking density was 100 PL/decimal or less (Fig. 6). Stocking frequency was more than twice in Bagerhat region compared to the other two districts.
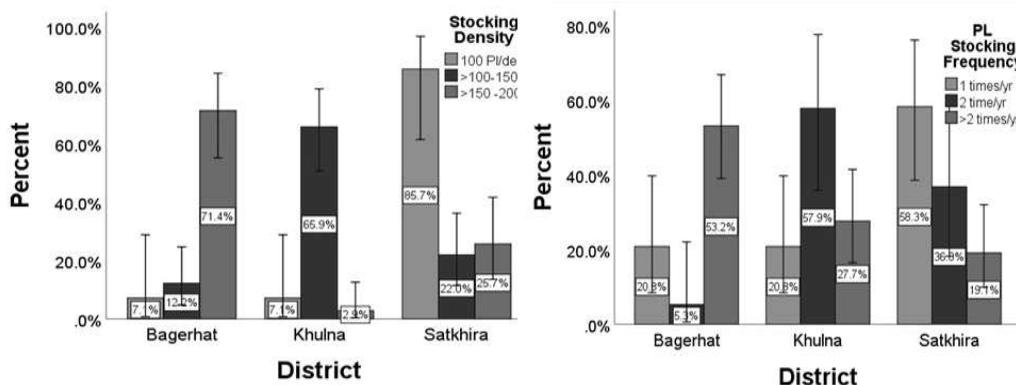
Fig. 6. Stocking density and PL stocking frequency of shrimp.

Post-stocking management
Mostly the formulated feed was used in maximum farms in Khulna and Satkhira regions instead of commercial feeds but in Bagerhat region commercial feed was used (Fig. 7). The amount of feed used in most of the ghers of the studied area was less than 5% of the body weight but 16.13% gher used feed at 10% or above 10% of body weight in Bagerhat (Fig. 7). The investigated farms were improved traditional farms under the cluster based farming system. Maximum farmers had given feed 1 to 2 times per day in

the studied area but some ghers of Satkhira and Khulna had given feed 3 to 4 times per day (Fig. 8). Regular monitoring of water quality was done in all the ghers of Bagerhat but in Satkhira water quality parameters were not checked in maximum farms (Fig. 8). To increase the primary productivity in most of the ghers of the studied area applied aqua drugs and prebiotics (Fig. 9). The culture period of most of the ghers in the studied area was maintained above 8 months but in Satkhira and Bagerhat region, culture duration of some ghers was above 6 to 8 months (Fig. 10). On the basis of our face to face questionnaire data collection, we have found the survival rate in maximum farms of Bagerhat and Khulna district was 60 to 70%. In Satkhira, the survival rate was above 70 to 80% (Fig. 10).
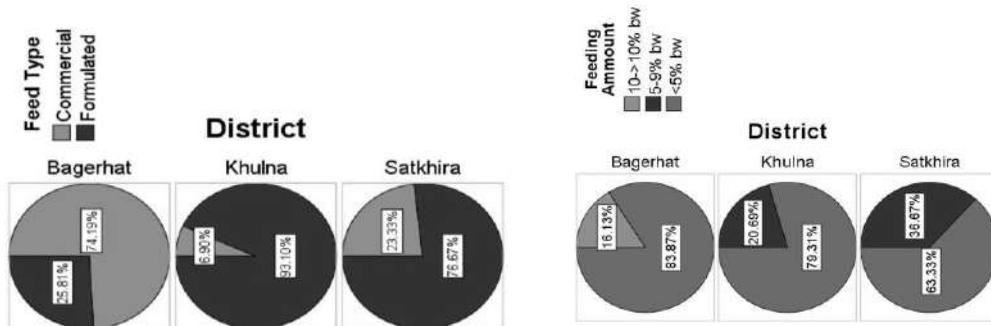
Fig. 7. Overview of using feed type and feeding amount in *ghers.*
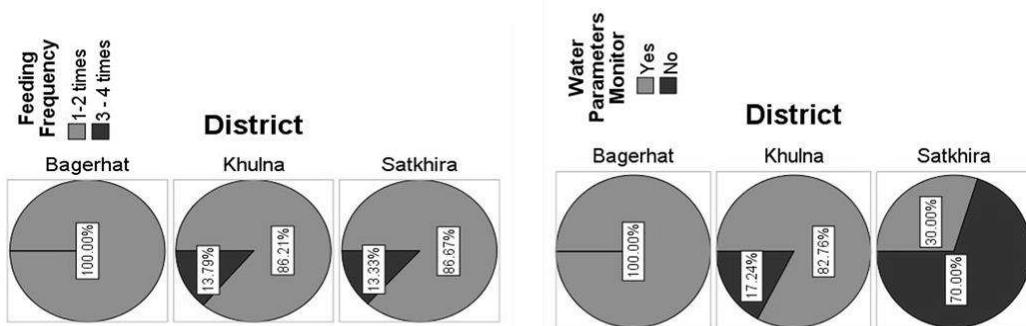
Fig. 8. Feeding frequency and water parameter monitoring status.
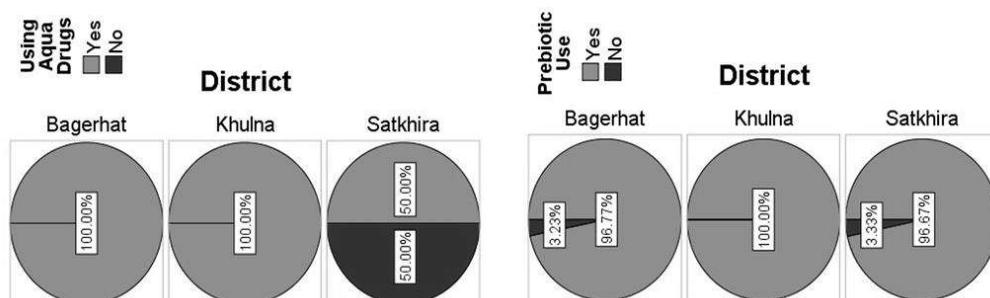
Fig. 9. Aqua drugs and prebiotic used status in *ghers.*
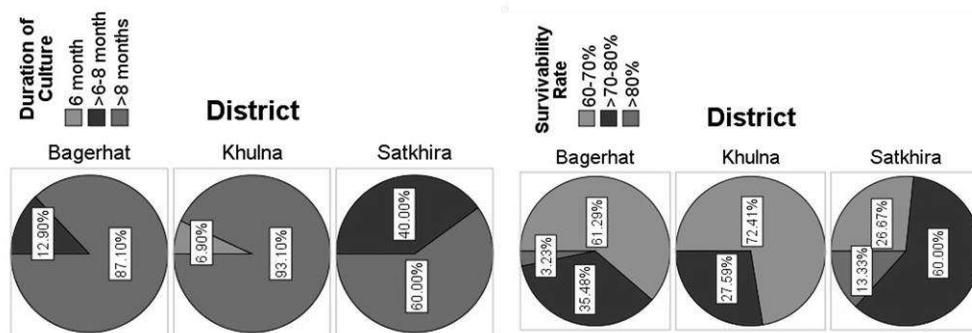
Fig. 10. Culture duration and survivability rate.

Discussion

Shrimp culture in tidal watercourse has been an ongoing practice in the Satkhira, Khulna, and Bagerhat districts of Bangladesh. As a result of the rising demand for shrimp on the international market, shrimp farming is quickly spreading horizontally at present. In Bagerhat maximum farmer had experience above 10 years but 50% of farmers had 1 to 5 years' experience in Khulna. On the other hand, most farmers had experience above 5 years to 10 years in Satkhira, which supported the previous findings of Chowdhury *et al.* (2007) who observed that 80% of farmers started shrimp farming in 2000-2004 in the Southern region of Bangladesh. Haque *et al.* (2023) found that 57.73 percent of the surveyed shrimp farms were between 1.01 and 2.99 hectares in size which were quite larger than the gher size of the present study. The average depth of the gher in the rainy season was slightly higher than that in the dry season, but 80% of the ponds had an average depth of <3 ft which was similar to the findings of Chandra *et al.* (2013). Shawon *et al.* (2018) explored water supply canal availability for the intake and drainage of water

to the ghers of the Khulna district which was also identified in the present study. The water source of ghers in Bagerhat and Khulna region was fully dependent on rivers except Satkhira where 10% of ghers are dependent on the ground water which coincides with the findings of Al-Mamun *et al.* (2020). Water exchange was done in most of the ghers which was also quite similar to the findings of Akter and Khan, (2021). Pond preparation was done methodically for greater productivity and quality shrimp production (Akter and Khan 2021) which was also followed by the farmers of surveyed regions. Bari (2004) mentioned that about 45% of shrimp farmers used fertilizers in their farms but the present scenario was quite different because all surveyed farmers used fertilizer which indicated that fertilizer use had increased.

It was found that farmers cultured about 100%, 75.85 and 76.67% of shrimp with white fish in Bagerhat, Khulna, and Satkhira, respectively, whereas only 24.14% and 23.33% farms in Khulna and Satkhira regions cultured only shrimp (Tiger shrimp and freshwater giant prawn) i,e. monoculture system which was similar to Chandra *et al*. (2010). In Bagerhat most of the PL used in the culture system was hatchery produced SPF which is similar to the findings of Kabir (2009) and Al-Mamun *et al.* (2020). The average stocking density of the study was found 175 individuals/dec; 120 individuals/dec and 100 individuals/dec in Bagerhat, Khulna, and Satkhira respectively, except Bagerhat, which was almost similar to Thangadurai (1991), who reported the density 120 individuals/dec. McGinty and Alston (1993) recommended a 100 individuals/dec stocking density for shrimp farming Kabir (2009) observed PL stocking in the farms in the coastal region of Bangladesh 4-5 times/year which was more than the present finding. Only in the Bagerhat region farmers release PLs more than 2 times which was similar to Kabir (2009) but most of the farmers of Khulna & Satkhira release PLs one time or two times.

Feeding is vital in aquaculture because fish growth is dependent on the quality of feed. Different types of feeds are widely used in aquaculture. Almost all farms in the present study used either formulated or commercial feed but only supplemental feed was used in the farms of Khulna district (Shawon *et al*. 2018). Aqua drugs were used in most of the farms for disease treatment, aqua clean, and used as antibiotics (Akter and Khan 2021, Matin *et al.* 2016) but in the present study, most farmers used prebiotics to increase the total production of shrimp. Measurement of water quality parameters is important for all aquaculture systems. The majority of farmers took water quality measurements (Akter and Khan 2021, Shamsuzzaman and Biswas 2012) which was similar to the present study where the maximum farmers of Bagerhat and Khulna districts measured water quality parameters. Culture duration is dependent on the farmer's desire. In the present study, most of the farmers of coastal regions had cultured white fish with shrimp for more than 8 months.

The survival of shrimp in the present study was higher than the previous findings (Alam and Phillips 2004, Karim and Stellwagen 1998). Alam and Phillips (2004) observed that the large farms in the outer category had the highest shrimp survival rate (45%), whereas individual and group farmers had 24% and 26% shrimp survival rate, respectively. Karim and Stellwagen (1998) observed that the stocked post larvae had survived 20%.

Conclusions and Recommendations

The main income source of the southwest coastal farmers of Bangladesh is shrimp farming, which plays an emperative role in their way of life. Most farmers in coastal regions started small-scale shrimp farming a few decades ago. Farmers are now engaging in larger-scale shrimp culture practices. However, the production and price of shrimp in our country is comparatively lower than other nations. Production, can be increased up to two to three folds by enhancing management procedures and culture systems. Therefore, to improve the traditional shrimp farming system and ensure the long-term prosperity of rural shrimp farmers, the authorities should be concerned about the import of virus-infected stocks, disease surveillance, and the dissemination of new scientific knowledge among farmers.

Although shrimp culture technology is being improved day by day in the Southwestern part of Bangladesh, this study provides some recommendations based on the problems faced by the farmers to improve shrimp production further.

- Root-level farmers should be helped to get the legal market value of shrimp as the low market value can threaten the total culture method.
- The Government should provide healthy, and SPF PLs for better production.
- The government and private sector or the NGOs should come forward to establish more shrimp seed multiplication farms and hatcheries in the study area to increase the production of good quality shrimp seeds, so that the farmers can get the shrimp seed easily and at a cheap rate.
- Approved aquatic drugs should be required against the severe and most common disease of shrimp, White Spot Disease.

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Reproductive biology, feeding habits, and conservation perspectives of Gangetic Whiting, *Sillaginopsis panijus*: A review

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Abstract

Sillaginopsis panijus, commonly known as the Gangetic Whiting, is a commercial fish species inhabiting the diverse ecosystems of the of the estuarine water. This review synthesizes existing knowledge on the breeding biology, feeding habits, and conservation aspects of *S. panijus*, aiming to provide a comprehensive understanding of its ecological role and conservation needs. Through a thorough analysis of available literature, this paper elucidates the morphological characteristics, distribution patterns, reproductive biology, feeding behavior, and habitat preferences of *S. panijus*. Additionally, it examines the importance of this species and discusses various conservation challenges and strategies pertinent to its sustainable management. The findings underscore the importance of integrated conservation efforts to safeguard the ecological integrity and long-term viability of *S. panijus* populations of the estuarine water.
Keywords: *Sillaginopsis panijus*, Gangetic Whiting, Reproductive biology, Feeding habits

Introduction

Gangetic whiting, *Sillaginopsis panijus* sometimes known as "Tular dandi", is a commercially valuable fish species that plays vital ecological roles in the region's estuary and coastal ecosystems. *S. panijus* makes significant contributions to the region's artisanal and commercial fisheries, providing an essential source of protein for coastal populations while also serving as a commercial target (Azim *et al.* 2012). The Flathead Sillago, *S. panijus* (Hamilton 1822), is a demersal inshore marine and estuarine fish that lives in Bangladesh, India, Myanmar, Malaysia, and the Indonesian archipelago. It is in high demand in both local and distant markets, and its white meat and lack of intramuscular bones make it a delicacy (Islam *et al.* 2012).

A recent study found that *S. panijus* populations are rapidly falling throughout Bangladesh's southern coast as a result of overfishing, the introduction of exotic species, pollution, damming, and even the effects of global climate change (Siddik *et al.* 2015).

Despite its ecological and socioeconomic importance, investigations on the biology of the Flathead Sillago are scarce, with very few scientific materials available globally (Islam *et al.* 2012, Hossain *et al.* 2015, Siddik *et al.* 2015, 2016a). Understanding the fisheries biology, eating habits, feeding habitat preferences, and conservation needs of *S. panijus* is critical for optimal management and sustainable use of this resource in the Bengal Delta ecosystem. This study seeks to close this information gap by integrating existing literature on several aspects of *S. panijus* biology and ecology, with a focus on feeding behavior, habitat preferences, and conservation status of the estuarine water.

Materials and Methods

This review was compiled by doing a thorough search of academic databases like as PubMed, Google Scholar, and Web of Science using keywords such as "*Sillaginopsis panijus*," "morphometric-meristic," "reproductive biology," "feeding habits," "conservation," and "Bengal Delta." This review looked at relevant articles, research papers, reports, and books that were published between 1980 and 2024. The selected literatures were thoroughly examined to extract relevant information on the physical traits, distribution, reproductive biology, feeding ecology, habitat preferences, fishing trends, and conservation status of *S. panijus* in the Bengal Delta.

Habitat and Distribution

A migratory amphidromous fish, the *S. panijus* is found in the Gangetic delta, Pondicherry (Coromondoal coast), Bangladesh (Siddik *et al.* 2015), Burma-Malaysia, and, on occasion, Indonesia (Hamilton 1822, Talwar and Jhingran 1991, Rahman, 2005, Azim *et al.* 2012). Both the western Pacific Ocean and the Indian Ocean are home to a large population of this family. This species of fish migrates to the higher sections of tidal rivers to extend their habitat for reproducing and in quest of food. It is an estuary and inshore marine fish that has adapted to the muddy substrate in shallow water (Hamilton 1822, Talwar and Jhingran 1991). This kind of fish, known as a Sillaginid, is found across Bangladesh's marine and coastal waters, with a preference for shallow, muddy bays (Rahman 1989). The Gangetic delta, the southwest coast, the coast of Bangladesh (Hanif *et al.* 2015a), some regions of India, Myanmar, and Malaysia, as well as the Indonesian archipelago on occasion, are among the locations where *S. panijus* is found (Azim *et al.* 2012). Despite its extensive geographical distribution, this species is found in greater abundance in Bangladesh's southwest coastal rivers and estuaries than anywhere else (Hanif *et al.* 2015b). Throughout the Padma Delta in Bangladesh, Myanmar, and India's Pondicherry, as well as occasionally in the Indonesian Archipelago, is the demersal inshore marine and estuarine fish *S. panijus* (McKay 1992).

Morphometric and Meristic Characters

The most common methods for defining populations of fish species that are commercially fished are morphometric and meristic parameters (Murta 2000, Silva 2003, O'Reilly and Horn 2004). According to Franicevic *et al.* (2005), morphological features are frequently variable amongst species that originate from various geographical locations. Variable body shapes indicate variable growth, death, or reproduction rates of the stock. Morphological variances convey stock characteristics (Swain and Foote 1999, Cadrin 2000, Hossain *et al.* 2006). These characteristics are crucial for the management and conservation of overfished fisheries resources because they are the most widely used and reliable methods for characterizing the stock, identifying fish assemblages, and differentiating between populations (Cheng *et al.* 2005, Siddik *et al.* 2016a, b, Hossain *et al.* 2012). This species is also known as the "Flathead Sillago" because of its extremely lowered head, tiny eyes that are constrained by orbits, and severely modified second spine out of the ten on the first dorsal fin (McKay 1992). The sillaginids' homogeneous body forms make them simple to identify. The most popular and economical method for characterizing fish species is thought to be morphological identification (Cadrin and Silva 2005, Chaklader *et al.* 2015, Sidik *et al.* 2021).

According to Rahman (1989, 2005) from Bangladesh, *S. panijus* could reach a maximum length of 27.5 cm. Indian researchers like Talwar and Jhingran (1991) also noted that *S. panijus* might grow up to be 44 cm long. According to Pradhan *et al.* (2020), the maximum total length measured in the coastal waters of the northwest Bay of Bengal was 40.3 cm. For males, the length range was 11-33.2 cm, and for females, it was 9.9-40.3 cm. The total length of *S. panijus* from Bangladeshi waters varied between 17.4-34.0 cm (Hossain *et al.*, 2015) and 14.6-29.3 cm (Siddik *et al.* 2015). Islam *et al.* (2012) recorded a maximum length of 38.2 cm from Bangladesh's Meghna Estuary. 44.0 cm is the longest length that has been reported (Talwar and Jhingran 1991). Total length (TL) varied from 11.5 to 34.5 cm for men and from 11.5 to 37.0 cm for females, according to Sabbir *et al.* (2022). Body weight (BW) varied between 6.79 to 225.57 g for male and 10.57 to 368.49 g for female population. Islam *et al.* (2012) also documented the variation in Total Length (cm). *S. panijus's* total length was 36.04±0.87 cm in August and 36.08±0.69 cm in September. August had the lowest value while September had the greatest value. With each passing month, *S. panijus's* overall length grew. There were no discernible variations between the mean total length (36.06±0.52 cm) and the total length measured in two different months.

Monalisa and Chanda (2021) studied *S. panijus* from Rupnarayan River, West Bengal, India. They observed shiny reddish silver color in the anterior, whitish silver in the abdomen, with pale brownish fins and black dusting spots. The body shape of this fish elongated with a small mouth, lateral eyes, small teeth and the whole body covered small ctenoid scales and a lateral line with 91 to 93 scales. The dorsal fins are well-separated, with elongated spines extending to the caudal fin. The paired fins have 19 to 22 rays, the anal fin has two spines and 25 to 27 soft rays, and the caudal fin has 18 to 20 rays. The opercula have a well-developed spine.

Pradhan *et al.* (2020) reported fin formula with gill raker count derived from an analysis of meristic counts was D1 X, D2 I + 24-28, P 17-22, V I + 5, A II + 25-27, GR 2-3/6-9. Islam *et al.* (2012) reported dorsal fin spines (10), dorsal fin soft rays (27-28), pectoral fin rays (16-20), pelvic fin spines (1), pelvic fin soft rays (5), anal fin spines (2), anal fin soft rays (24-28), caudal fin rays (17-19), scale on lateral line (83-98), scale above lateral line (6-9) and scale below lateral line (10-15). The first dorsal fin contains nine spines including a large trailing second spine; the second dorsal fin has one spine and 27 to 28 soft rays. McKay (1992) reported for *S. panijus* the dorsal spines (total): 11; dorsal soft rays (total): 25-27; anal spines: 2; anal soft rays: 24-27; scales on lateral line: 84–90; vertebrae: 42. Islam *et al.* (2012) provided data on fin and scale counts for the species: dorsal fin spines (10), dorsal fin soft rays (27-28), pectoral fin rays (16-20), pelvic fin spines (1), pelvic fin soft rays (5), anal fin spines (2), anal fin soft rays (24-28), caudal fin rays (17-19), scale on lateral line (83-98), scale above lateral line (6-9), and scale below lateral line (10-15). The first dorsal fin comprises nine spines, including a large trailing second spine; the second dorsal fin has one spine and 27 to 28 soft rays. Pradhan *et al.* (2020) described the fin formula and gill raker count as D1 X, D2 I + 24-28, P 17-22, V I + 5, A II + 25-27, GR 2-3/6-9. McKay (1992) documented key features for *S. panijus* including dorsal spines (total): 11; dorsal soft rays (total): 25-27; anal spines: 2; anal soft rays: 24-27; scales on lateral line: 84–90; vertebrae: 42.

The Gangetic whiting was described by Rahman (1989, 2005) as having two dorsal fins, with the second spine of the first dorsal fin being particularly elongated and filamentous. The fin formula was also displayed, and it was D1. IX; D2. I/26-27; P. 23-24; P2. 6 (1/5); A. II/25-26; S. 90-93. A II 24-26; P 24; V I 5; S. 84-88; and the morphology of *S. panijus* were also noted by Talwar and Jhingran (1991). Sabbir *et al.* (2021) found *S. panijus* to have an elongated body with a depressed head and snout, two dorsal fins with elongated second spine, small eyes nearly covered by fleshy orbits, forked caudal fin, and greenish yellow dorsum with pale underside. Fins were pale brownish with light black spots. Total length ranged from 8.5-37.2 cm (mean: 19.75±6.25 cm) and body weight from 6.46-285 g (mean: 56.58±59.24 g). The species exhibited both spiny and soft fin

rays. Fin formulas included First Dorsal, D1 IX; Second Dorsal, D2 I/24-28; Pectoral, P.17-20; Pelvic Pv.1/5; Anal, A I-II/24-27; and Caudal, C 2/16-18. The lateral line contained 82-86 scales. Islam *et al.* (2012) noted changes in body weight in August and September, two distinct months. September had the lowest body weight (252.23 ± 37.40 g) while August had the highest (348.48 ± 10.97 g). Siddik *et al.* (2016) conducted a study in three coastal rivers of Bangladesh namely Meghna, Tentulia and Baleshwar and found significant morphometric differences of *S. panijus.*

Length-weight Relationship

In case of ecological assessment, monitoring, and management, length-weight relationships (LWRs) are essential (Sinovcic *et al.* 2004, Froese, 2006). The use of LWRs is less time-consuming and useful to determine weight from length and vice versa because fisheries data collection systems having some restrictions (Pauly 1993). To evaluate the biomass using analytical methods like cohort or virtual population analysis (VPA), the length class mean weight estimate is crucial (Andrade and Campos, 2002; Morey *et al.,* 2003). Additionally, LWRs can be used to compare the population over time and space, as well as to estimate the number of fish that are landed (Beverton and Holt 1957, Thomas *et al.* 2003). It's employed to distinguish between populations of the same species in various settings (Goncalves *et al.* 1997). The length-weight relationship scatter plot, which is shown differently for *S. panijus* males, females, and indeterminates, was described by Pradhan *et al.* (2020). The male, female, and indeterminate b values were determined to be 3.059, 3.185, and 3.0645, respectively. These values indicate a positive allometric growth pattern within the species. In the Gangetic Delta of Bangladesh, allometric growth was recorded for females (b = 3.209) and pooled sex (b = 3.096), and negatively allometric growth was recorded for males (b = 2.994) (Siddik *et al.* 2015). Similar results, with b values reported as 3.48 and 3.30, respectively, were also noted by Islam *et al.* (2012) in the Meghna River Estuary, Bangladesh, and Hossain *et al.,* (2015) in the Tetulia River, southern Bangladesh. The differences in the sampled size ranges brought about by the exploitation of various gears are responsible for this variance in *b* values. According to Sabbir *et al.* (2022), positive allometric growth was shown for both sexes by the allometric coefficient (b) value (male, 3.14; female, 3.18). 0.0056 was the computed form factor (*a 3.0*). It was discovered that Fulton's condition factor (KF) was best suited for this species.

Reproductive Biology

Sex Ratio: Understanding the fish sex ratio is crucial for ensuring that fishing efforts are balanced between the sexes and for determining a population's capacity for

reproduction (Vazzoler 1996). Understanding the relationships between people, their surroundings, and the status of the population requires knowledge of the sex ratio (Vicentini and Araújo 2003). Many factors, including population adaptation, reproductive behavior, food availability, and environmental conditions, can cause the sex ratio to deviate from the expected 1:1 across species or even within the same population at different times (Nikolsky 1963, Emlen and Oring 1977, Baroiller and Cotta 2001, Brykov *et al.* 2008, Vandeputte *et al.* 2012). For *S. panijus*, reproductive data, including the sex ratio, is essential to the preservation and administration of biological resources. The overall sex ratio (male: female) was calculated by Pradhan *et al.* (2020) to be 1:8.2, which clearly indicates that the population is predominately female. This ratio deviates from the expected ratio of 1:1. On the other hand, Siddik *et al*. (2015) found that the study's sex ratio was 1:1.17. According to Sabbir *et al.* (2022), the total sex ratio is 1.00:0.78 for males and females.

Fecundity: Fish fecundity is the term used to describe a female fish's ability to reproduce or the quantity of eggs she produces during the spawning season. To evaluate *S. panijus's* reproductive capacity, population dynamics, and the sustainability of the fisheries resources of the estuarine water, it is essential to comprehend its fecundity. In fisheries, fecundity is a crucial indicator of a stock's capacity for reproduction. A fish's fecundity assessment is crucial for assessing its life history, practical culture, commercial potential, and actual fishing management (Mohamad *et al.* 2020). According to Rahimibashar *et al.* (2012), estimating a fish's fecundity and GSI is crucial for assessing the fishery's real management as well as its life history, practical culture, and commercial potential. Islam *et al.* (2012) found that *S. panijus* had a mean fecundity of 310,605.1±22,833.84, with a range of 173,745 to 374,077. Total length (36.06±0.52 cm), body weight (300.36±24.41 g), and gonad weight (28.65±2.98 g) were all linked with fecundity. A fish that measured 34.6 cm in total length, weighed 155.05 g, and had a gonad weight of 11.51 g had the lowest fecundity (173,745), while a fish that measured 38.2 cm, weighed 378.22 g, and had a gonad weight of 38.89 g had the highest fecundity (374,077). In most cases, the right lobe was larger than the left when it was ripe. Islam *et al.* (2012) also revealed that during the breeding months (August-September), there were some variances in the fecundity levels. August had the highest level of fecundity (339017.81±14676.71), while September had the lowest number (282192.47±41520.15). In two months, a declining tendency in *S. panijus* gonad weight was observed. August had the highest value (34.74±1.55 g) while September had the lowest value (22.56±4.34 g). Islam *et al.* (2012) claimed variation in the fecundity of the fish in the same length class was found in their study which indicates that the fecundity of a fish is not solely dependent on its length. Fish measuring 38.2 cm in total length, 378.22 g in body weight and 38.89 g in gonad weight produced 374,077 eggs, whereas another fish of the same

total length 38.2 cm, body weight 345.43 g and gonad weight 31.3 g produced 366490 eggs. Apart from scientific investigations, environmental elements including food availability, water temperature, and habitat quality could also have an impact on *S. panijus* fertility. For example, research has demonstrated that environmental variables, such as temperature shifts brought on by climate change, can affect fish species' ability to reproduce, particularly their fecundity.

The gonadosomatic index (GSI): The gonadosomatic index (GSI) is a commonly used indicator of fish maturity, gonadal development, and level of reproductive investment in relation to spawning. It is defined as gonad mass as a percentage of total body weight (Quyyam and Quasim 1961, Bagenal and Tesch 1978, Fagbenro *et al.* 1991, Cubillos and Claramunt 2009). Fish gonadosomatic index rises with maturity and sharply drops following spawning. Given that the female gonads enlarge before spawning, GSI is especially useful in determining the season of spawning (Biswas 1993, Idowu 2017). Research on fish reproductive biology is crucial for both conservation efforts and the selection of wild fish candidates for aquaculture (Muchlisin 2013). A unique and diversified aquatic ecosystem, the Bengal Delta is formed by the confluence of the Ganges, Brahmaputra, and Meghna rivers and is home to a wide range of fish species, including *S. panijus*. Understanding the reproductive patterns of *S. panijus* facilitates research on its GSI, which benefits conservation and sustainable fisheries management initiatives. Research on *S. panijus's* GSI yields important data on the species' spawning season, fecundity, and reproductive capacity—information that is crucial for developing fisheries management plans. According to Islam *et al.* (2012), the GSI for *S. panijus* had an average value of 9.95±0.15% in August and 8.67±0.58% in September. A declining GSI value denotes a declining gonad development from August to September, indicating August as the peak season. It is common knowledge that as fish mature, the gonadosomatic index rises. It peaks at peak maturity and then sharply declines.

Spawning season: For the purpose of managing the local fisheries and promoting conservation, it is essential to comprehend its spawning season. In marine fishes, spawning behavior is linked to resilience (Lowerre-Barbieri *et al.* 2017) and productivity (Cheung *et al.* 2005). As a result, the temporal and spatial aspects of spawning may have an impact on the relationship between stocks and recruitment, which in turn may have an impact on how a species reacts to fishing pressure (Maunder and Deriso, 2013; Donahue *et al.,* 2015; Erisman *et al.* 2017). For example, enhanced reproductive resilience is positively connected with both the number of spawning locations and opportunities (Erisman *et al.* 2011, Lowerre-Barbieri *et al.* 2015). The length of the spawning season is inversely correlated with vulnerability; species that spawn year-round or over extended seasons tend to experience slower and less severe population

declines than those that have predictable but shorter spawning periods (Mullon *et al.* 2005, Claro *et al.* 2009, Sadovy De Mitcheson and Erisman 2012). The spawning season of *S. panijus* of the estuarine water typically coincides with specific environmental factors, particularly water temperature, salinity, and photoperiod. While exact timing may vary slightly depending on local conditions, several studies have shed light on the general timing of their spawning activities. The juveniles of *S. panijus* migrate into the upper section of the tidal river between the months of December and March-April after the species spawns twice a year (most likely in August–September and November–February) (Talwar and Jhingran 1991). Twice a year, from August to September and from November to February, *S. panijus* spawns (McKay 1992). At a length of 12 cm, this oviparous species (Breder and Rosen, 1966) reaches sexual maturity (McKay 1992). Additionally, this species is amphidromous, meaning it can move between freshwater and the ocean, and it can withstand extreme climatic conditions (Sabbir *et al.* 2021a).

Feeding habits: Fish feeding behavior is miscellaneous and has been broadly examined in both wild and farmed fish from their ecological perspectives (Gerking 2014). while behavioral responses of fish to feeding have been associated with feeding approaches, feeding habits, feeding regularity, feed detection mechanisms and feed preferences (Lall and Tibbetts 2009). Despite the species' importance along the Indian coast, especially in the northwestern Bay of Bengal, nothing is known about its feeding environment. Fish, algae, and crustaceans are the preferred dietary items (Mookerjee *et al.* 1946). This species typically consumes algae, tiny fish, and crustaceans (McKay 1992).

Nutrient composition

Azim *et al.* (2012) studied seasonal variations in the composition of Gangetic Sillago, finding crude protein as the main component, peaking at 73.93% in April and dropping to 57.79% in December. Carbohydrate content peaked at 9.31% in December and decreased to 1.31% in April, showing significant seasonal variation ($p<0.05$). Lipid percentage decreased in summer but slightly increased in the monsoon. The dry matter percentage remained consistent across all seasons.

Conservation aspects

Although the status of *S. panijus* is Least Concern in Bangladesh according to IUCN Bangladesh (2015), conservation efforts must focus on preserving the integrity of its habitats, including mangrove forests, mudflats, and shallow coastal waters, which serve as crucial breeding and feeding grounds for the species. Overfishing and pollution are some of the major threats for the *S. panijus* populations. Furthermore, sustainable

fishing practices, such as implementing mesh size regulations and seasonal fishing bans, are necessary to prevent overexploitation of *S. panijus* populations.

As a major predator in estuarine and coastal food webs, *S. panijus* is essential to the ecology and socioeconomics of the estuarine water, providing support for artisanal fishing and subsistence farming. The sustainability of *S. panijus* populations is threatened by increasing anthropogenic pressures and habitat degradation, which calls for immediate conservation actions. Through an explanation of the reproductive biology, eating habits, and conservation issues associated with S. panijus, this paper highlights the significance of integrated management strategies for enhancing the species' ability to adapt and remain resilient in the face of environmental change. The conservation and sustainable use of *S. panijus* resources of the estuarine water Delta and elsewhere depend on cooperative initiatives that prioritize habitat preservation, ethical fishing methods, and community involvement.

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